Pharmacogn J. 2025; 17(1): 99-106
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

In Vitro Antibacterial Activity, *In-Silico* Molecular Docking and Pharmacokinetic Profile of a Phytosterol Isolated from The Leaves of *Carissa Bispinosa*

Wanda Shekwa, Mashilo Mash Matotoka, Tsolanku Sydney Maliehe, Peter Masoko*

ABSTRACT

Carissa bispinosa (L.) Desf. Ex-Brenan is a medicinal plant widely used in South Africa. The study reports on the isolation of a phytosterol from *C. bispinosa* along with molecular docking and pharmacokinetic studies. The phytochemicals were extracted with hexane, dichloromethane, acetone and methanol. The compound was isolated following a bioactivity-guided isolation protocol using column-chromatography and thin-layer chromatography. Nuclear magnetic resonance (NMR) was used for compound characterisation. The antimicrobial activity was assessed using bioautography and micro-broth dilution assays. AutoDock vina, SwissADME and ADMET lab were used for molecular docking, pharmacokinetic and toxicological properties, respectively. Drug-likeness was evaluated based on Lipinski's rule of five (Ro5). The isolated compound was identified as β-sitosterol. It had an anti-*S. aureus* activity of (0.31 mg/mL). It revealed binding scores of -7.2 and -6.4 kcal/mol against penicillin-binding protein and DNA gyrase, respectively. It violated one of Ro5 (MLOGP > 4.15). It has no inhibitory effects against isoforms of cytochrome P450. Moreover, it demonstrated no adverse effect on skin, non-carcinogenic and non-hepatotoxic effects. Based on the results, β-sitosterol can be considered as potential anti-*S. aureus* drug. Further studies may focus on ascertaining the mechanism of action *in-vitro* and investigating the synergistic effects of the compound with conventional drugs.

Keywords: *Carissa bispinosa,* Antimicrobial, Pharmacokinetics, Molecular docking.

Wanda Shekwa, Mashilo Mash Matotoka, Tsolanku Sydney Maliehe, Peter Masoko*

Department of Biochemistry, microbiology and Biotechnology, University of Limpopo, Private bag X1106, Sovenga 0727, SOUTH AFRICA.

Correspondence

Peter Masoko

Department of Biochemistry, microbiology and Biotechnology, University of Limpopo, Private bag X1106, Sovenga 0727, SOUTH AFRICA.

E-mail: peter.masoko@ul.ac.za

History

- Submission Date: 29-12-2024;
- Review completed: 21-01-2025;
- Accepted Date: 04-02-2025.

DOI : 10.5530/pj.2025.17.14

Article Available online

http://www.phcogj.com/v17/i1

INTRODUCTION

Infectious diseases continue to be the major cause of death worldwide despite the availability of antibiotics. The persistent emergence of antibiotic-resistant bacteria, adverse effects of antibiotics, the emergence of new infectious diseases for which no treatment exists are the driving forces of this calamity [1,2]. By 2050, the predicted annual worldwide mortality rate due to antimicrobial resistance (AMR) might rise to 10 million, a significant rise from the present estimate of 700,000 deaths annually.[3] This underscores the importance of finding alternative antimicrobial agents with different mechanisms of action.

The conventional medication discovery and development processes are complicated. It takes about 10 – 15 years for a drug to be clinically approved and the cost is exorbitant, over 1 billion USD.[4] Although most of newly discovered lead compounds are found to be pharmacologically efficacious, about 90% of them fail during the process of drug development due to lack of desirable drug likeliness properties, toxicity concerns and poor pharmacokinetics.[5] These lead compounds fail to demonstrate the standard physicochemical properties set by different drug filters such as Lipinski`s rule of five (Ro5) to be regarded as druglike compounds. Moreover, their pharmacokinetic properties, which are describe by their absorption, distribution, metabolism, and excretion (ADME) characteristics or better ADMET when toxicity studies are included, tend to be unacceptable.[6] In addition, conventional evaluation of these properties is time consuming, tedious and costly.[7]

Recent developments in artificial intelligence (AI) play a crucial role in expediting the drug development process. A variety of *in silico* techniques for virtual screening compounds from virtual chemical spaces offers improved profile analysis, quicker elimination of nonlead compounds, and more cost-effective therapeutic molecule selection.[8] AI or computational techniques such as molecular docking, molecular dynamics simulation and *in silico* ADME studies are widely used to streamline the drug discovery road.[9] Molecular docking is used to predict protein-protein and drug-protein interactions, while ADME analyses the pharmacokinetics of potential drugs. Molecular dynamics simulations decipher the movement and behaviour of proteins and molecules in a biological setting.[10] Although computational methods are not confirmatory, they do provide information of the most likely drug-like compounds out of an array of compounds.

Natural products and their derivatives have a wide range of biological activities and are potential sources of novel drugs.[11] Approximately 50% of currently used pharmaceuticals and nutraceuticals are natural products or derived from natural sources.[12] Medicinal plants play a pivotal role in primary health care and may be the source of novel drugs.[13] Most rural communities especially in the African continent heavily rely on traditional medicine due to their accessibility, low cost, lower side effects and their holistic approaches.[14] Phytochemicals have the potential to inhibit the growth of bacteria,

Cite this article: Shekwa W, Matotoka MM, Maliehe TS, Masoko P. *In Vitro* Antibacterial Activity, *In-Silico* Molecular Docking and Pharmacokinetic Profile of a Phytosterol Isolated from The Leaves of *Carissa Bispinosa*. Pharmacogn J. 2025;17(1): 99-106.

fungus, viruses, and protozoa through distinct mechanisms compared to currently available antimicrobials. This makes them potentially valuable in the clinical treatment of resistant microbial strains.[15]

Carissa bispinosa is an evergreen plant and is one of approximately 85 species belonging to the carissa genus under the Apocynaceae.[16] The genus is found throughout Oceania, Africa, and Asia. Due to its long history of usage in traditional medicine, the carissa genus enjoys a significant ethnobotanical.[17] Traditional applications of *C. bispinosa* (Umvhusankunzi in Zulu) include treating toothaches with the roots and stimulating male libido with the stem, according to a traditional healer in Mabandla Village, Umzimkulu, KwaZulu-Natal, South Africa.[18] Within the carissa genus, *C. bispinosa* is the least investigated species, and little is known about the plant's bioactive components.[17] *C. bispinosa*'s leaf and stem extracts (dichloromethane, acetone, and methanol) had antibacterial action against *S. aureus, E. faecalis, S. pyogenes*, and *C. albicans*.[19] *S. aureus, E. faecalis,* and *C. albicans* showed susceptibility to the acetone root extract.[18]

Elucidating the mechanism of action, mechanisms of bacterial resistance, and isolating the bioactive compounds are highly relevant and important subjects for research in validating the use of medicinal plants and/or searching for novel antimicrobial agents.[20] The isolation of an antibacterial compound from *C. bispinosa* leaf extracts is reported in this paper, along with the computational analysis of its molecular interactions with antimicrobial targeted receptors. Moreover, the *in silico* physicochemical properties, drug-likeness and ADMET properties of the isolated compound were also predicted.

METHODS

Media and chemicals

The analytic grade chemicals and culture media were acquired from Sigma-Aldrich, Whitehead Scientific, Adcock-Ingram, and Merck (Pty) Ltd. respectively. The water used in the study was autoclaved for 15 minutes at 121 degrees Celsius.

Microbial strains

Oral isolates from Polokwane Hospital, National Health Laboratory Service (NHLS), South Africa, were utilised in this investigation. The fungal (*Candida albicans* and *Candida glabrata*) and bacterial (*Streptococcus pyogenes, Staphylococcus aureus,* and *Enterococcus faecalis*) strains were obtained from this facility.

Plant collection

The plant's leaves were collected on July 8, 2019, at 1335 meters above sea level in the Limpopo province of South Africa, at latitude -23.885425 S and longitude E. The University of Limpopo's research ethics committee approved the acquisition of the plant, and the voucher specimens were added to the University of Limpopo Larry Leach Herbarium (UNIN) for future reference (UNIN 1220078). The current study used plant extracts in accordance with international guidelines.[21] The leaves were cleaned with tap water to get rid of dirt and debris, and then they were allowed to air dry in the absence of heat and light to preserve the structures of compounds that are sensitive to heat. The dried plant components were ground into a powder using an electric grinder (Sundy hamercrusher SDHC 150) and then kept until needed in a dark plastic bag made of polypropylene.

Serial exhaustive extraction

Six litres of each solvent: n-hexane, dichloromethane, acetone, and methanol were used sequentially and thoroughly to extract from a mass of 0.6 kg leaf powder. The solvents were used in order of non-polar to polar. In the first batch, each solvent was allowed to shake with the plant material at 200 rpm for an entire night (I), then for two hours (II), and finally for one hour (III). Using a Buchi B-490 rotary evaporator, the extracts were filtered and concentrated before being transferred to 250 mL beakers. Using room temperature cold air, the residual solvent was evaporated.

Thin-layer chromatography

The phytochemical profile of the extracts was produced using TLC plates with an aluminium backing (Merck, silica gel 60 F254). Ten microliters of the extracts were spotted on the TLC plate. Three solvent systems were used to develop the plate: ethyl acetate/methanol/water (40:5:4.5): [EMW] (polar/neutral); ethyl acetate/chloroform/formic acid (5:4:1%). [BEA] (non-polar/basic); benzene/ethanol/ammonium hydroxide (90:10:1): [CEF] (intermediate polarity/acidic);[22] On the TLC plates, 0.1 g of vanillin (Sigma ®): 28 mL methanol: 1 mL of concentrated sulfuric acid reagent was sprayed. The plates were then heated for two minutes at 110 °C to achieve the best colour development.

Screening of the antimicrobial activity of the extract

Culture preparation

The microbial isolates were inoculated in 150 mL flasks containing Sabouraud dextrose broth (fungi) and nutrient broth (bacteria) and incubated at 30 and 37 °C, respectively. The bacterial and fungal suspensions were adjusted to a density of 1 × 108 CFU/mL−1.

Bioautography assay

A bioautography assay was used to test the antimicrobial activity of the extract.[23] To completely evaporate the solvents, the plates were allowed to air dry at room temperature for three to five days. After being sprayed onto the plates, the test microorganisms were incubated for 24 hours at 37 °C and 100% relative humidity. The chromatograms were incubated for approximately two to three hours after being sprayed with an aqueous solution containing 2 mg/mL of ρ-iodonitrotetrazolium chloride (INT) (Sigma).

Isolation of the active compound

Column chromatography

Four open columns chromatography systems were packed with silica gel 60 (particles size 0.063 – 0.200 mm) (Fluka) were used to further fractionate the active extract. The 1st column (39 x 3.5 cm) was eluted with gradient elution of n-hexane, ethyl acetate and methanol, sub-fractions 5 – 10 were subjected to the 2nd column (38 x 3.5 cm) and eluted with isocratic elution of n-hexane: ethyl acetate (1:1). Active fraction (1-7) from the 2nd column were pooled and further fractionated on the 3rd column (31 x 4 cm) using n-hexane: ethyl acetate (70:30). The 4th column was separated the sub-fractions 1 – 2 using n-hexane: ethyl acetate (70:30) as an eluant.

Identification of the compound

The fourth open-column chromatography sub-fractions (test tubes 90 – 160) were combined and separated using BEA on TLC silica gel glass plates (Merck Silica gel 60 F254). The compound of interest was traced using the Rf value as a reference and then scraped off the developed TLC plates. The compound was submerged in ethyl acetate and filtered through cotton wool to remove the silica. The isolated compound was then identified using nuclear magnetic resonance (NMR).

Antibacterial activity of the isolated compound

Bacterial stock cultures were prepared by inoculating the fungal and bacterial culture into a 150 mL Sabouraud dextrose liquid media and nutrient broth and incubated at 30 and 37 °C, respectively. A hundred microlitres of the compound was serially diluted to 50% with distilled

water in 96 well microtitre plates. About 100 µL of the compound was added into the first well and diluted serially with microbial media. Microbial cultures (100 µL) were added to each well. Acetone was used as a solvent control and gentamicin was used as a positive control. For negative control, only the test organisms and media were added to the wells. The microtitre plates were covered with a plastic wrap (Glad) and incubated at 30 and 37 °C for 24 hours, respectively, to allow for microbial growth. After incubation, 40 µL of (0.2% w/v in water) ρiodonitrotetrazolium chloride (INT) (sigma) was added to each well as an indicator. All the tests were performed in triplicates, and the results are represented as the mean of the three values.[24]

Molecular docking

Preparation receptor proteins and ligand

Molecular docking was done to predict the mechanism of antimicrobial activity of the isolated compound. Penicillin-binding protein (PDB ID: 3HUN) and DNA gyrase (PDB ID: 3G7B) were used in this investigation. The proteins were obtained from the Protein Data Bank (PDB) (www.rcsb.org). Using the Discovery Studio software version 4.1, the proteins were optimised to improve effective docking by removing heteroatoms, water molecules, and other ligand groups before adding polar hydrogens. The three-dimensional (3D) structure of isolated compound (β-sitosterol) was downloaded from the National Centre for Biotechnology Information (NCBI) PubChem compound database.[25]

Docking of the prepared ligand and receptors

AutoDock Vina was used to carry out the *in silico* molecular docking studies to ascertain the mechanism of antimicrobial activity of the isolated compound. The binding spheres for 3HUN (-33.648125, 13.629896 and -10.162354 for the x, y, and z centers) and 3G7B (27.830552, -5.584207 and 8.598638 for the x, y, and z centers) were found from the receptors' active sites using the Discovery Studio 4.1. The binding scores of the ligand-receptor complexes were predicted using AutoDock.[26] The ligand-receptor complexes with the lowest binding energy scores were considered to have the best-docked conformations. The generated two and three-dimensional (2D and 3D) conformations were then visualized by importing the docked complexes into Discovery Studio version 4.1.

Physicochemical properties, lipophilicity and solubility prediction

The physicochemical properties, lipophilicity and solubility of the isolated compound were computed using Swiss ADME online tool.[27] Briefly, the simplified molecular-input line-entry (SMILES) of the isolated compound was retrieved from PubChem and inserted into the Swiss ADME. Thereafter, the physicochemical properties including the molecular weight, number of hydrogen bond donors (nHBD), number of hydrogen bond acceptors (nHBA) and number of rotatable bonds (nRB) were evaluated. Lipophilicity was predicted using consensus logP (clogP) and solubility (LogS) was assessed using the ESOL, Ali and SILICOS-IT models.

ADMET properties prediction

The pharmacokinetic properties of the isolated compound were evaluated using Swiss ADME and the toxicity was calculated using ADMET lab online tool.[27] The pharmacokinetics profile entails Absorption, Distribution, Metabolism and Excretion (ADME). The SMILES of the compound was inserted to Swiss ADME tool to predict pharmacokinetic parameters such as gastro-intestinal absorption (GIA), blood-brain barrier (BBB) penetration, interaction of the compound with P-glycoprotein (P-gp) and isoforms of cytochrome P450 (CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4). For toxicological studies, the SMILES were deposited to the ADMET lab tool which calculated the carcinogenic, human hepatotoxicity (HHT) and skin sensitivity (Skin Sen) of the compound.

Drug-likeness and bioavailability predictions

The Swiss ADME tool was used to investigate the drug-likeness of the compound [27], whereby Lipinski's rule of five (Ro5) was used as a criterion. According to Ro5, for the isolated compound to be desirable, it must not violet more than one of Ro5, which filters based on molecular weight ≤ 500 Da, log P-value ≤ 5, nHBD ≤ 5, nHBA ≤ 10 and nRB ≤ 10. The bioavailability of the compound, which refers to the extent it can be completely available to its intended biological destination(s), was predicted based on MV, cLogP, nHBD and nHBA.

RESULTS

Antimicrobial activity of the extracts

Figure 1, shows the bioautograms of the extracts against the test microorganisms. The active compound was detected on the dichloromethane and acetone extracts developed in the non-polar BEA mobile system as shown by the white bands against a pink background. More vivid bands were observed against *S. aureus*. The lowest minimum inhibitory concentration was observed on the dichloromethane extract (I) against *S. aureus* (0.31 mg/mL) as shown in table 1.

Isolation and identification of the compound

The compound was identified as β-sitosterol based on its physical properties (white crystal) and colorimetric analysis with TLC-spray reagent (development of a blue-violet color). The NMR data of the isolated compound was found to be similar to β-Sitosterol (Table 2 and Figure 2).

Antibacterial activity of the compound

The compound had a minimum inhibitory concentration of 0.31 mg/mL.

Molecular docking

The computed molecular docking results demonstrated that β-sitosterol had better binding affinities against penicillin-binding protein (-7.2 kcal/mol) than DNA gyrase (-6.4 kcal/mol). Gentamicin which was used as a positive control had binding scores of -6.6 and -6.2 kcal/mol against penicillin-binding protein and DNA gyrase respectively. At least one hydrogen bond was observed for all the ligand-receptor complexes. Figure 3, shows all the interactions between the ligands and the residues of the selected receptors. β-sitosterol formed hydrogen bonds with ASN 138 and alkyl bonds with LYS 249 and PHE 243. On DNA gyrase, it interacted with TYR 141, forming a hydrogen bond. It also illustrated van der Waals forces. Gentamicin interacted with ARG 214, GLU 224, GLU 224 and THR 212 through hydrogen bonding. It also formed carbon hydrogen bond with GLU 224 and GLU 224. Against penicillin binding protein, gentamicin formed hydrogen bond with ASN 117 and ASN 138; it revealed carbon hydrogen bond with SER 75.

Physicochemical properties and lipophilicity

β-sitosterol was found to possess the following physicochemical properties, molecular weight of 414.71 g/mol, number of hydrogen bond donors (1), number of hydrogen bond acceptors (1) and number of rotatable bonds (6). Lipophilicity indicator, consensus logP (clogP) predicted a value of 7.19.

Solubility

The solubilities of the compound in aqueous solution were -7.90 for Log S (esol), -9.67 (Log S Ali) and -6.19 (Log S SILICOS-IT) classes.

Table 1: MIC values (mg/mL) of *Carissa bispinosa* extracts, gentamicin (Gent) and amphotericin-B (Amp) against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococcus faecalis* Candida albicans and *Candida glabrata*.

Microorganisms	MIC (mg/mL)													
	n-Hexane			Dichloromethane			Acetone			Methanol			Gent	Amp
	I	II	III	I	II	III	I	II	III	I	II	III		
S. aureus	1.25	0.63	0.63	0.31	1.25	1.88	0.63	1.25	1.25	1.25	2.5	2.5	0.08	
E. faecalis	1.25	0.63	0.94	0.94	1.25	1.25	0.63	0.94	1.25	1.88	2.5	1.88	0.04	
S. pyogenes	1.25	0.63	0.94	0.31	1.25	1.25	0.63	1.25	1.88	2.5	1.88	1.88	0.04	
C. albicans	1.25	1.25	0.94	0.47	0.94	1.25	0.94	1.88	0.94	1.88	2.5	2.5		0.08
C. glabrata	1.88	1.25	1.25	0.94	1.25	1.25	0.94	1.25	1.25	2.5	2.5	2.5		0.08

Table 2: 1H, 13C NMR spectral data of isolated compound and β-sitosterol from literature.

Position	Type	β-sitosterol [28]		Isolated compound	
		Chemical Shift, δ (ppm) value			
		13C NMR	1 H NMR (multiplicity	13C NMR	1H NMR (multiplicity)
1	CH_2	37.28	1.46 (m)	38.77	1.65-1.60 3H
2	CH_2	31.69	1.56 (m)	30.5	191-1.85 3H
3	CH(OH)	71.82	3.54 (m)	79.01	3.21 (m)
4	CH_2	42.33	2.32 (m)	41.92	2.32 (m)
5	QC (=)	140.77	-	137.87	-
6	CH(=)	121.73	5.37 (overlapping, t)	125.79	5.23 t (J= 3.5 Hz)
7	CH_2	31.93	2.04 (m)	31.91	2.15 (m)
8	CH	31.93	1.69 (m)	31.91	2.12 (m)
9	CH	50.16	1.55 (m)	47.86	1.91-1.85
10	QC	36.51	-	36.66	-
11	CH_2	21.11	1.52 (m)	21.16	1.65-1.60
12	CH_2	39.80	1.51 (m)	9.41	1.65-1.60 8H
13	QC	42.34	-	41.57	-
14	CH	56.79	1.50 (m)	55.13	1.65-1.60 8H
15	CH_2	24.33	1.58 (m)	23.55	1.74-1.67
16	CH_2	28.27	1.85 (m)	27.94	1.99 (m) 2H
17	CH	56.08	1.45 (m)	52.57	1.65-1.60 8H
18	CH_3	11.89	0.70 (s)	15.29	0.91 (s)
19	CH_3	19.42	1.03 (s)	15.44	1.12 (s)
20	CH	36.17	1.60 (m)	36.94	1.74-1.67 3H
21	CH_3	18.84	0.94 (overlapping, d)	15.57	1.06
22	CH_2	33.98	0.93 (m)	32.88	0.89 (m) 2H
23	CH_2	26.11	1.15 (m)	27.15	1.52-1.46 (m) 4H
24	CH	45.86	1.38 (m)	47.4	1.58 (m) 1H
25	CH	29.19	1.57 (m)	24.35	1.74-1.67
26	CH_3	19.84	0.84 (overlapping, d)	18.24	0.91 (s)
27	CH_3	19.06	0.86 (d)	17.00	0.97 (s)
28	CH_2	23.10	1.10 (m)	22.68	1.52-1.46 4H
29	CH_3	12.01	0.82 (overlapping, t)	14.12	0.86 (s)
-	OH	-	1.98 (s)		

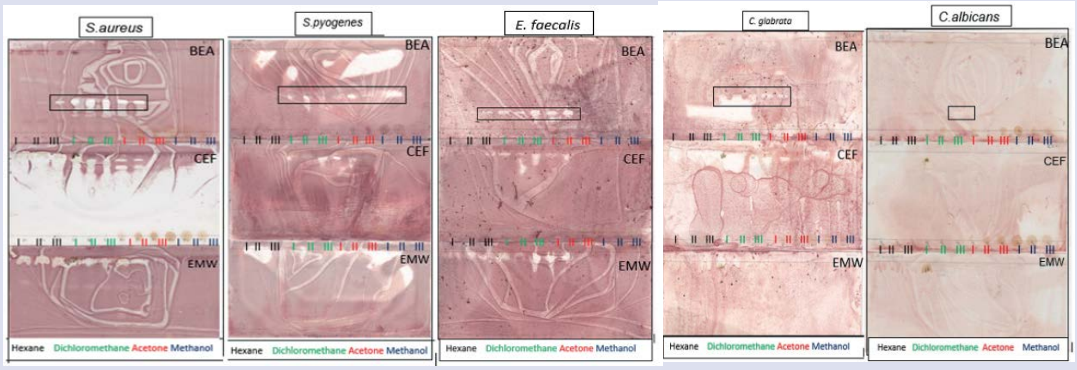


Figure 1: Bioautograms of *C. bispinosa*'s leaf extracts developed in BEA, CEF and EMW mobile systems. The chromatograms were sprayed with 24-hour culture of *S. aureus, S. pyogenes, E. faecalis, C. albicans* and *C. glabrata.*

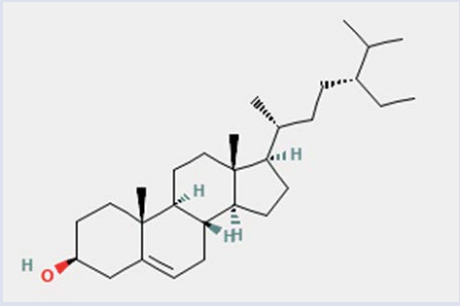


Figure 2: Structure of the isolated compound (β-sitosterol).

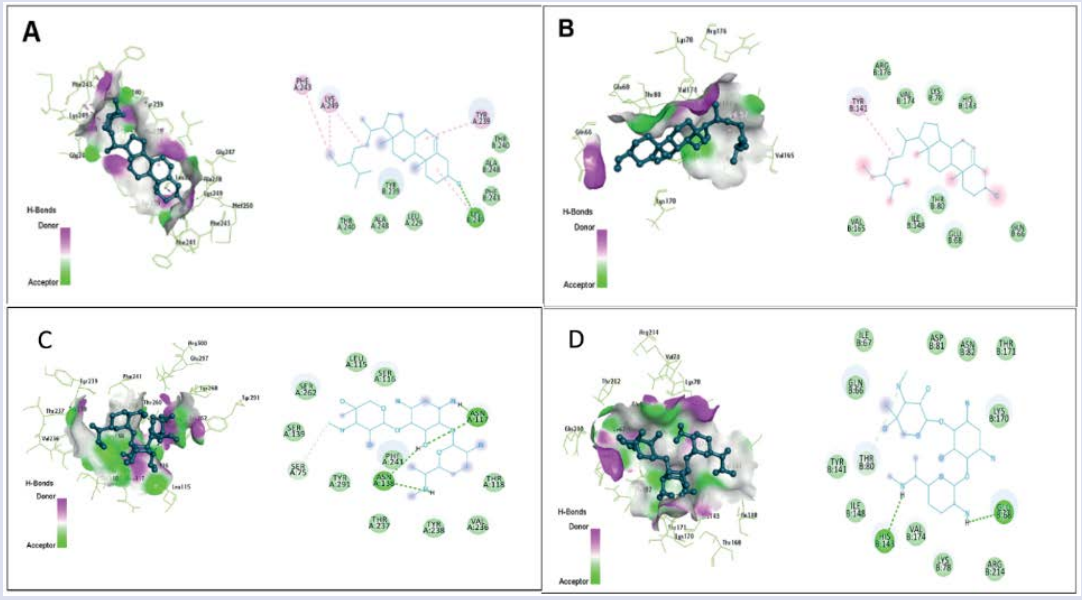


Figure 3: 3D (left) and 2D (right) showing the interactions of β-sitosterol and with (A) penicillin-binding protein, (B) DNA gyrase and gentamicin against (C) Penicillin binding protein and (D) DNA gyrase.

Drug likeness and bioavailability

The compound violated only one of lipinski' rule (MLOGP ≤ 4.15). The bioavailability of the compound was 0.55.

Pharmacokinetics

The compound had a low gastro-intestinal absorption and is not a permeant of the blood-brain barrier. Furthermore, β-sitosterol is not a substrate of p-glycoprotein and demonstrated no inhibitory effects against the isoforms of cytochrome P450 (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4). The LogKp was found to be -2.2 cm/s.

Toxicological properties

On toxicological evaluation, it showed no hepatotoxicity, carcinogenicity and no effect on skin sensitivity.

DISCUSSION

Isolation of bioactive compounds is a significant aspect in the search for new therapeutic agents. In this study, an antibacterial compound was isolated from the leaves of *C. bispinosa* and its in silico drug-likeness, molecular interactions with target receptors, and ADMET studies were predicted. The bioautograms revealed zones of inhibition of dichloromethane and acetone crude extracts against *S. aureus, E. faecalis, S. pyogenes* and *C. albicans* on the BEA mobile phase, inferring that the bioactive compound is non-polar. The dichloromethane extract had moderate activity against *S. aureus* and *C. albicans* (0.31 mg/mL) and the acetone extracts had poor to moderate activity against all the tested pathogens. The MIC is considered significant if (MIC <100 µg/mL), moderate (100 ≤ MIC ≤ 625 µg/mL) and low (MIC > 625 µg/mL).[29]

The active extracts were pooled together and fractionated using open column chromatography. Four column chromatography systems and preparative-TLC led to the isolation of a white crystal compound (12 mg). The Rf value (1. 17) of the active bands on the bioautograms corresponded with the Rf value of a blue-violet band on the TLC fingerprint (BEA mobile system) after spraying with vanillin sulphuric acid reagent. The development of a blue-violet color upon spraying with vanilin sulphuric acid reagent is a characteristic of triterpenes and steroids [30]. NMR analysis identified the compound as β-sitosterol with reference to the work reported by Ododo et al.[28]. β-sitosterol exhibited moderate antibacterial activity (0.63 mg/mL) against *S. aureus*. Ododo et al.[28] also reported anti-*S. aureus* activity. Another study reported antimicrobial activity of β-sitosterol against S. aureus and other bacterial (*Pseudomonas aureginosa, Corynebacterium ulcerans and vulgaris*) and fungal (*Candida virusei* and *Candida albicans*) pathogens.[31]

Molecular docking simulation was performed *in silico* to predict the mechanism of antibacterial activity employed by β-sitosterol. The

compound was docked against penicillin-binding protein (PDB ID: 3HUN) and DNA gyrase. Penicillin binding proteins (PBP) are involved in the biosynthesis of the cell in bacteria while DNA gyrase is responsible for the negative superhelix required for chromosome replication.[32,33] Therefore, these protein receptors are the ideal targets for antibacterial compounds. During molecular docking, proteins become less disordered when the entropy decreases, which makes protein–ligand complexes more stable during interaction.[34] Based on the results in the current study, the most stable complex was the β-sitosterol-penicillin binding protein. The complex was stabilised by the strong hydrogen bond between the ligand and the receptor at ASN A 138, 2.29 residue; the binding of functional groups of β-sitosterol to LYS 249 and PHE 243 further strengthened the interactions. This suggests that the compound may act on penicillin-binding protein to elicit an anti-*S. aureus* activity.

Lipinski's rule of five (Ro5) is widely used to predict the drug likeliness of lead compounds. Ro5 filters are based on the following physicochemical properties: molecular weight ≤ 500, MLOGP (lipophilicity) ≤ 4.15, hydrogen bond acceptors ≤ 10, and hydrogen bond donors ≤ 5. A lead compound must not violate more than one of the Ro5.[35] β-sitosterol only violated the clogP ≤ 5 rule hence, it can be considered a drug lead. A high degree of lipophilicity may negatively affect solubility, selectivity, potency, permeability and promiscuity of a drug.[36]

Lipophilicity (clogP) regulates the solubility, potency, selectivity, permeability and promiscuity of druglike compounds. High lipophilicity (clogP > 5) results in lead compounds with high rapid metabolic turnover, poor solubility and absorption. β-sitosterol revealed high clogP value, consequently violating one of the Ro5. The compound` solubilities using logS model, predicted it to be poorly soluble in aqueous solution. The poor solubility was attributed to the undesirable clogP value, which did not adhere to the Ro5. Some drugs can be administered through the skin depending on their physicochemical properties, LogKp measures the ability of a compound to permeate the skin. A negative LogKp value was observed in this study, indicative of the inability of β-sitosterol to permeable through the skin. This means that β-sitosterol is not suitable for transdermal administration.[37] This implied that β-sitosterol can be administered through other routes rather than dermal. Moreover, its inability to penetrate through the skin indicates biosafety against its effect during skin contact.

Bioavailability of an oral administered drug measures the rate at which a compound is circulated by the blood system to the target site, the compound had a bioavailability score of 0.55, indicating 55% probability of the compound to reach the target sites.[38] For an oral administered drug to reach its target areas, it must be absorbable in the gastrointestinal tract and distributed to the desired location.[39] β-sitosterol had a poor gastrointestinal absorption (GIA) value, which also indicated a disadvantage for oral administration. Distribution predictions include blood–brain barrier (BBB) permeability. BBB is the endothelial cell membrane which regulates the ability of a drug to permeate from the blood stream to the brain. β-sitosterol cannot pass through the BBB membrane, implying that the compound cannot be used to treat brain related infections and the central nervous system. BBB permeability is only required for drugs that act on the central nervous system .[40] Moreover, the inability of the compound to penetrate through the BBB suggested that the compound cannot pose any adverse effects to the brain. P-glycoprotein (P-gp) belongs to the ATP-binding cassette transporters (ABC transporters) superfamily. These proteins transport molecules across a cell membrane against a concentration gradient, resulting in multidrug resistance.[41] β-sitosterol is not a substrate of P-gp, suggesting that the compound has ability to exert its therapeutic actions at targeted sites without been affected by the expression of P-gp. The isoforms of cytochrome P450 (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4) play a major role in the metabolism of drugs. Inhibition of these enzymes results in drug-drug interactions due to the accumulation of the drug and its metabolites. β-sitosterol was predicted as a non-inhibitor to all selected isoforms. This meant that the compound has high probabilities of being metabolised and eliminated from the system after exerting its activities at respective target sites.[42] The compound exhibited no carcinogenicity, hepatotoxicity and no effect on skin sensitivity. This suggested that β-sitosterol can be administered without causing any severe negative side effects.

CONCLUSION

The study was the first to report the isolation of β-sitosterol from the leaf extracts of *C. bispinosa*. The isolated compound was predicted to exert its antibacterial activity against *S. aureus* by interacting with the penicillin binding proteins and DNA gyrase. β-sitosterol was shown to be a lead compound due to its bioactivity and drug-likeness. The pharmacokinetics of β-sitosterol suggested the compound to have desirable properties as it did not illustrate any potential to inhibit cytochrome P450 isomers and is not a substrate of P-gp. The toxicity predictions revealed the compound to have a high margin of biosafety as it did not reveal any toxic effects. Further studies are needed for structural modification of the compound to enhance its oral adsorption, solubility, and lipophilicity. Moreover, focus on the synergistic effects of β-sitosterol and known antibiotics against the tested pathogens is suggested.

ACKNOWLEDGEMENTS

We would like to thank the University of Limpopo for offering a platform to carry out this study. Dr Ofentse Mazimba for his assistance with structural elucidations and Ms Tselane Ramakadi for her assistance with the generation of the NMR spectra of the sample.

AUTHOR CONTRIBUTIONS

WS and PM contributed to the study's conceptualization and design. WS conducted the experiments, examined the data, and drafted the paper. TSM and MMM made editing and revisions to the paper. The final manuscript was read and approved by every author. The final manuscript was read and approved by each author.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article

COMPETING INTERESTS

There is no conflict of interest.

REFERENCES

1. AlSheikh HMA. et al. Haq QMR Plant-based phytochemicals as possible alternative to antibiotics in combating bacterial drug resistance. Antibiotics. 2020;9(8):480.
2. Bandow JE, Brotz H, Leichert I. Proteomic approach to understanding antibiotic action. Antimicrob Agents Chemother. 2003; 47:948–55.
3. Globally TD. An overview of our work/the review on antimicrobial resistance chaired by Jim O'Neill. Globally TD. 2016.
4. Lexchin, J. Models for financing the regulation of pharmaceutical promotion. *GHJ*. 2012; 8:24.
5. Sun D, Gao W, Hu H, Zhou S. Why 90% of clinical drug development fails and how to improve it? Acta Pharmaceutica Sinica *B*. 2022; 12(7):3049-62.

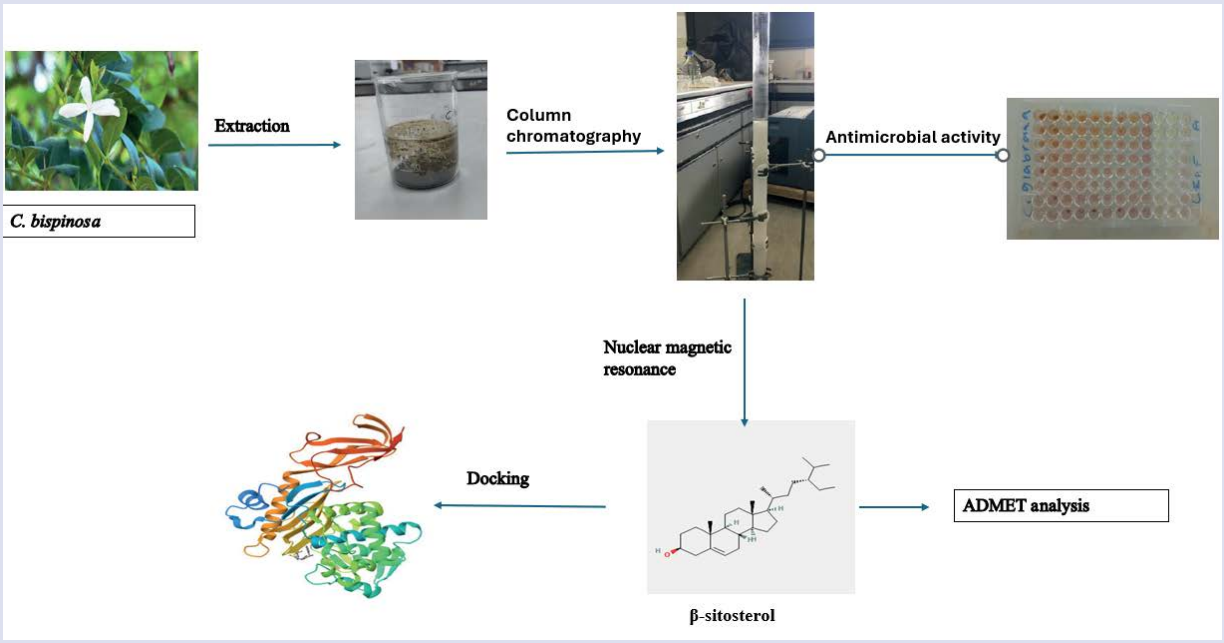
6. Dearden, J.C. *In silico* prediction of drug toxicity. J Comput Aid Mol Des. 2003; 17:119–27.
7. Venugopala, K.N. *et al*. Anti-tubercular potency and computationally-assessed drug-likeness and toxicology of diversely substituted indolizines. Indian J Pharm Educ. 2019; 53:545–552.
8. Mak KK, Pichika MR. Artificial intelligence in drug development: present status and future prospects. Drug Discov Today. 2019; 24(3):773–780.
9. Hussen NH. *et al*. Potential inhibitory activity of phytoconstituents against black fungus: *In silico* ADMET, molecular docking and MD simulation studies. Comput Toxicol. 2022; 24:100–247.
10. Hollingsworth SA, Dror RO. Molecular dynamics simulation for all. Neuron. 2018; 99(6):1129-1143.
11. Nathwani D, Raman G, Sulham K, Gavaghan M, Menon V. Clinical and economic consequences of hospital-acquired resistant and multidrug-resistant *Pseudomonas aeruginosa* infections: a systematic review and meta-analysis. Antimicrob Resist Infect Contr. 2014; 3:32.
12. Chavan SS, Damale MG, Devanand B, Sangshetti JN. Antibacterial and antifungal drugs from natural source: A review of clinical development. Natural products in clinical trials. 2018; 1:114–164.
13. Jamshidi-Kia F, Lorigooini Z, Amini-Khoei H. Medicinal plants: past history and future perspective. J Herb Med Pharmacol. 2018; 7:1–7.
14. Schenone M., et al. Target identification and mechanism of action in chemical biology and drug discovery. Nat Chem Biol. 2013; 9:232–240.
15. Shankar, S.R., Rangarajan, R., Sarada, D.V.L. & Kumar, C.S. Evaluation of antibacterial activity and phytochemical screening of *Wrightia linctoria* L. Pharmacogn J. 2010; 2:19–22.
16. Lindsay EA, Berry Y, Jamie JF, Bremner JB. Antibacterial compounds from *Carissa lanceolata* R Br. Phytochemistry. 2000; 55:403–406.
17. Dhatwalia, J. *et al*. Phytochemistry, pharmacology, and nutraceutical profile of Carissa Species: An Updated Review. Molecules. 2021; 22:7010.
18. Muleya E, Ahmed AS, Sipamla AM, Mtunzi FM. Free radical scavenging and antibacterial activity of crude extracts from selected plants of medicinal value used in Zululand. Pak J Nutr. 2014; 13(1):38.
19. Shekwa W, Maliehe TS, Masoko P. Antimicrobial, antioxidant and cytotoxic activities of the leaf and stem extracts of *Carissa bispinosa* used for dental health care. BMC Complement Med Ther. 2023; 23(1):462.
20. Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E. Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. Microorganisms. 2021; 9(10):2041.
21. World Health Organization. WHO guidelines on good agricultural and collection practices [GACP] for medicinal plants. World Health Organization. (2003).
22. Kotze M, Eloff JN. Extraction of antibacterial compounds from *Combretum spp*. (Combretaceae). 2002; S Afr J Bot. 68:62–67.
23. Begue WJ, Kline RM. The use of tetrazolium salts in bioautographic procedures. J Chromatogr A. 1972; 64:182–184.
24. Pfaller MA, Andes D, Diekema DJ, Espinel-Ingroff A, Sheehan D. CLSI subcommittee for antifungal susceptibility testing. wild-type MIC distributions, epidemiological cutoff values and species-specific clinical breakpoints for fluconazole and Candida: time for harmonization of CLSI and EUCAST broth microdilution methods. Drug Resist Updat. 2010; 13(6): 80–195.
25. Afriza D, Suriyah W, Ichwan SJA. *In silico* analysis of molecular interactions between the anti-apoptotic protein survivin and dentatin, nordentatin, and quercetin. J Phys Conf Ser. 2018; 1073(3): 032001.
26. Trott O, Olson AJ. Autodock vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comp Chem. 2010; 31:455–46.
27. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017; **7**:42717.
28. Ododo MM, Choudhury, MK, Dekebo AH. Structure elucidation of β-sitosterol with antibacterial activity from the root bark of *Malva parviflora*. SpringerPlus. 2016; 5:1210.
29. Bisso N, Kayoka-Kabongo PN, Tchuenguem RT, Dzoyem JP. Phytochemical analysis and antifungal potentiating activity of extracts from loquat (*Eriobotrya japonica*) against *Cryptococcus neoformans* clinical isolates. Adv Pharmacol Pharm Sci. 2022; 1:6626834.
30. Tamaka K. Phytochemical Investigation of *Caralluma attenuata* (Wight) Roots.
31. Reyes AWB. *et al*. β-sitosterol contributes in the resistance to invasion and survival of *Brucella abortus* 544 within RAW264.7 cells, and cytokine production with reduced susceptibility to infection in BALB/c mice. J Microbiol Biotechnol. 2020; 30:482–489.
32. Mohamed SB. *et al*. Proteomics and docking study targeting penicillin-binding protein and penicillin-binding protein 2a of methicillin-resistant *Staphylococcus aureus* Strain SO-1977 isolated from Sudan. Evol Bioinform. 2019; 15:1176934319864945.
33. Erol M, Celik I, Kuyucuklu G. Synthesis, molecular docking, molecular dynamics, DFT and antimicrobial activity studies of 5-substituted-2-(p-methylphenyl) benzoxazole derivatives. J Mol Struct. 2021; 1234:30–151.
34. Cong Y. *et al*. Alanine scanning combined with interaction entropy studying the differences of binding mechanism on HIV-1 and HIV-2 proteases with inhibitor. J Biomol Struct Dyn. 2021; 39, 1588–1599.
35. Lipinski CA. *et al*. and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 1997; 2:3–25.
36. Walker MA. Novel tactics for designing water-soluble molecules in drug discovery. *Expert Opin.Drug. Discov*. 9, 1421–1433. (2014).
37. Zhao H. *et al*. Mechanism study on ion-pair complexes controlling skin permeability: effect of ion-pair dissociation in the viable epidermis on transdermal permeation of bisoprolol. Int J Pharmaceut. 2017; 532(1): 29–36.
38. Singh S, Gupta AK, Chattree A, Verma A. Lead finding from whole plant of *curcuma longa* with oxidative potential. Int J Pharm Sci Res. 2013; 4(8):3142. (2013).
39. Heda P. *et al*. Identifying promising anticancer Sulforaphane derivatives using QSAR, docking, and ADME studies. JSR. 2021; 10(4).

40. Borra NK, Kuna Y. Evolution of toxic properties of antialzheimer's drugs through Lipinski's rule of five. Int J Pure App Biosci. 2013; 1:28–36.

41. Moitra K. Overcoming multidrug resistance in cancer stem cells. Biomed Res. Int. 2015; 635–745.

42. Szakács G, Váradi A, Özvegy-Laczka C, Sarkadi B. The role of ABC transporters in drug absorption, distribution, metabolism, excretion and toxicity (ADME–Tox). Drug Discov Today. 2008; 13:379–393.

GRAPHICAL ABSTRACT



Cite this article: Shekwa W, Matotoka MM, Maliehe TS, Masoko P. *In Vitro* Antibacterial Activity, *In-Silico* Molecular Docking and Pharmacokinetic Profile of a Phytosterol Isolated from The Leaves of *Carissa Bispinosa*. Pharmacogn J. 2025;17(1): 99-106.