NIPES

Journal of Science and Technology Research

Journal homepage: www.nipesjournals.org.ng

Theoretical Inquiry, Bioactive Characterization of Stigmasterol / β-Sitosterol from the Bulbs of *Calotropis procera* ('Sodom apple')

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Article Info

***Keywords**: Screening of Phytochemicals, Microbial bioassay, Calotropis procera, Characterization, ADMET and Molecular Docking*

Received 5 December 2024
Revised 28 March 2024
Accepted 31 March 2024
Available online 15 April 2024

https://doi.org/10.5281/zenodo.10971777

Abstract

This work characterizes sigmasterol from Calotropis procera bulbs and investigates the antimicrobial activities of the crude. The air dried pounded and sieved bulbs were extracted with 500 mL each of n-hexane, ethyl acetate and methanol respectively. Screening of the crude extracted in the above solvents revealed the presence of alkaloids, flavanoids, reducing sugars, saponins, steroids, and tannis. Anthraquinones were below detection level. Microbial bioassay was carried out against Nectricillin resistant Staphylococcus aureus, Vancomycin resistant Enterococci, Escherichia coli, Profeus mirabilis, Salmonella typhi, Pseudomonas aeruginosa, Candida albicans, Neisseria gonorrhea, Candida krusei and Candida stellafoidea. Ethyl acetate extract had the highest effective activity against Escherichia coli (MIC 2.5mg/mL and MBC/MFC 5mg/mL), Staphylococcus aureus (MIC 2.5mg/mL), Candida albicans, Salmonella typhi and Candida stellafoidea (MIC 5mg/mL). Extracts were subjected to column and thin layer chromatographic purification. The purified fractions were found to be white crystalline solids. Structures of the bioactive fractions were thoroughly elucidated using NMR spectroscopy, and comparison of their chemical shifts with literature confirmed the presence of the Stigmasterol and β-Sitosterol. Further studies revealed that the compounds possess profound anti-malarial, anti-ulcer, anti-tumour antifungal, antibacterial, analgesic and other pharmacological properties. Thus, this strong activity of the crude extracts against Escherichia coli and Salmonella typhi indicated that these molecules have anti-diarrheal activity. Molecular docking studies on the binding interaction between the DNA gyrase of Escherichia coli and Salmonella typhi revealed that the compounds majorly bind with the active sites of the target protease via hydrophobic and hydrogen bond interactions with binding activity of -7.8 kcal/mol, equivalent to the standard. In silico Drug-likeness and ADMET investigations of the compounds revealed their sound oral bioavailability, excellent pharmacokinetic and toxicity profiles. Thus, the bulbs of the P. procera are pharmacokinetically effective and efficient on above microorganisms and obey both the Lipinski and the Veber rules.

1.0. Introduction

Natural products have always served as a wellspring of inspiration for the field of drug discovery and successful source of future drug leads [1, 2, 3, 4 & 5]. It has been, it is, and will continue to provide unique structural diversity that presents opportunities for discovering mainly novel low molecular weight lead molecules [6]. Their significance extends to providing a deeper understanding of molecular properties, reactivity, and behaviour, impacting a diverse spectrum of fields, from foundational research to applications in environmental and materials sciences, [7]. Like most plants, biosynthesis of secondary metabolites derived from the processes of photosynthesis, produces precursors for the production of human remedies [8].

Calotropis procera a shrub or small tree is widely distributed in tropical and subtropical Africa [9]. It is known by different names like "Sodom apple, Usher, Dead Sea apple, Swallow-wort and Giant milk weed" [10]. The plant is hardy, pubescent, evergreen, erect, a compact shrub up to 4.5 m tall, and is covered with cottony tomentum. The stem is usually simple, rarely branched, woody at base and covered with fissures. Various parts of this plant have the ability to exude large quantities of latex when cut or broken [11]. *Calotropis procera (Asclepiadaceae)* is known to many traditional systems of medicine as 'Madar" in Unani medicinal system [12]. The Latex exuded by *Calotropis procera* is known for its high medicinal and pharmacological activity due to its high content of biologically active molecules such as; - cardiac glycosides, alkaloids, terpenes, resins, lipids, flavonoids, tannins and steroids [13]. This latex possesses different biological activities including: anti-inflammatory, analgesic, antitumor, antiviral, hepatoprotective, antiulcer, anthelmintic, insecticidal, herbicidal, antioxidant and spasmolytic activities [14].

Chemistry concepts such as *In silico* molecular docking, Pharmacokinetic and toxicity profiling has found immense applications in the discovery of new bioactive molecules for the treatment of various diseases [15, 16, 17 & 18]. Molecular docking entails the prediction of the binding interaction of bioactive compounds (ligands) with the active sites of a target macromolecule (receptor). The ligands with the most stable conformations with the target protein are the most promising drug candidates [19]. Molecular docking technique has been widely used in pharmaceutical research in recent years because of its fast and cost effectiveness in screening data based bioactive ligands [20, 21, 22, 23, 24, 25 & 17]. Likewise, pharmacokinetic investigation which is concerned with the fate of therapeutic ligands in the biological system forms an essential component of modern drug discovery. It deals with the absorption, distribution, metabolism, excretion, and toxicity (ADMET) potentials of bioactive ligands. *In silico* ADMET profiling of drug candidates helps to minimize attrition rates during the preclinical and clinical stages of drug development [26 &27]. Oral bioavailability (drug-likeness) prediction for therapeutic ligands is a fundamental evaluation in novel drug discovery and development owing to the fact that oral delivery remains the most common path of drug delivery into the systemic circulation. This study is aimed at the isolation, characterization and application of *in vitro* & *in silico* techniques to investigate the bioactive compounds, (Stigmasterol and β-Sitosterol) isolated in the Bulbs of *Calotropis procera*.

2.0. Materials and Method

Fresh bulbs of *Calotropis procera* were collected from Millionaires' Quarters, Lafia L. G A. Nasarawa State, in August, 2019 and authenticated at the College of Forestry and Fisheries, Federal University of Agriculture Makurdi, Benue State Nigeria with voucher No FH/0086, deposited at the College herbarium.

Cleaned dried fruits (5.0 kg) were then pulverized into coarse powder with mortar and pestle and stored in cellophane bags at room temperature until required for experiment use.

Fig. 1: Bulbs of *Calotropis procera*

2.1. Extraction

The plant material was weighed (500g) and extracted by Maceration allowing the pulverized powdered material to soak in a suitable solvent in a closed container at room temperature. Three selected solvents: N-Hexane, Ethyl Acetate and Methanol were used. The sample was macerated with two litres of each of the solvents for 72 hours with agitation. The mixture was filtered and the filtrate concentrated under reduced pressure using rotary evaporator at 37^{O}C, for solvent and extract recovery. This procedure was repeated for the ethyl acetate and methanol extracts. Recovery quantities were, 20.00g, of N-Hexane, 53.21g, of Ethyl Acetate and 80.26g of methanol respectively. The extracts were then allowed to dry and various solvents extracts were coded for quick identification.

2.2. Phytochemical Screening

Phytochemical screening was carried out for the presence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, reducing sugars, saponins, steroids, tannins, and terpenes, using standard procedures, [28 729]. Results of the phytochemical evaluation showed their presence.

2.3. Microbial Bioassay

Antimicrobial activities of the N- hexane, ethylacetate and methanol crude extracts were investigated using clinical isolates of some pathogenic microbes such as: *Staphylococcus aureus, Staphylococcus faecalis, Escherichia coli, Vancomycin enterococci, Neisseria gonorrhoae, Profeus mirabili, Pseudomonas aeruginosa, Salmonella typhi, Candida albicans, Candida krusei* and *Candida stellafoidea.*

Diffusion method was used for screening the extracts. Mueller Hinton Agar was used as the growth medium for microbes, sterilized at 121°C for 15 min, poured into Sterile Petri Dishes and allowed to cool and solidify. The crude extracts (0.4 g) were dissolved in 10 mL of Dimethylsulphoxide (DMSO) to acquire a concentration of 40 mg/mL. A sterilized medium was then seeded with the standard Inoculum (0.1 mL) of test microbes and spread evenly over the surface of the medium with sterile swabs. Using a 6mm standard cork-borer, a well was cut at the centre of each inoculated medium. A concentration of 5 mg/mL of the already weighed crude extract was then introduced into each well on the inoculated medium. The inoculated medium was incubated at 37^{O}C for 24 hours, after which the medium was observed for the zones of inhibition which were measured with a transparent ruler [30].

Minimum inhibition concentration, (MIC) of these extracts were carried out using Broth Dilution Method. Mueller Hinton Broth was prepared by dispensing 10 mL into test tubes and sterilizing at 37°C for 6 hours, then allowing to cool. McFarland's turbidity standard scale number 0.5 was prepared to give a turbid solution. Normal Saline (10 mL) which was dispensed into sterile test tubes and the test microbes were inoculated and incubated at 37^{O}C for 24 hours. Thereafter, the test tubes were observed for turbidity.
Thus, the lowest concentration of extracts in the sterile broth, which showed no turbidity, was recorded as the minimum inhibition concentration (MIC) [31]. The minimum bactericidal and minimum fungicidal concentration (MBC and MFC) were carried out to determine the concentration of extracts that could stop growth of test microbes. Mueller Hinton Agar was prepared, sterilized at 121^{O}C for 15 minutes, poured into sterile Petri dishes and allowed to cool. The contents of the test tubes with the determined MIC were then sub-cultured onto prepared media, incubated at 37^{O}C for 24 hrs, after which, the plates were observed for any colony growth. MBC/MFC plates with lowest concentration of extract, without a colony growth were considered as the MBC/MFC [32 &33].

2.4. Molecular Optimization and Docking Procedures

Microbial bioassay on the crude extract revealed that it possesses profound bioactivities against *Escherichia coli* and *Salmonella typhi*. Phytochemical screening revealed the presence of some secondary metabolites and spectroscopic characterization gave Stigmasterol and β-Sitosterol. The two ligands were subjected to geometry optimization using the Semi-empirical (pm3) method of Spartan'14 software to obtain their minimum energy geometries. Molecular docking simulation was used to screen the compounds against DNA gyrase of *Escherichia coli* and *Salmonella typhi*. The 3D structure of the target protease (PDB code: 5ztj) was retrieved from protein data bank at www. rcsb. org/pdb. The water molecules, hetero-atoms, and co-crystallized ligands attached to the retrieved protease were removed using the Biovia Discovery Studio interface. The target protein was further processed via the addition of Polar Hydrogens and Kollman charges with the aid of Auto Dock Vina tool v1.5.7. Finally, the docking calculations between the ligands and the target DNA gyrase was performed using PyRx software of Auto Dock Vina tool by centring the Vina search space at Y: 23.0598Å and Z: 22.0813Å with dimensions of Y: 53.0044Å and Z: 51.8136Å [34 & 35].

2.5. Drug-likeness Estimation

This is the evaluation of oral bioavailability of therapeutic compounds. Here, *in silico* technique forms a pivotal part of modern drug discovery owing to the fact that most drugs are administered via the oral route. The assessment of oral bioavailability of Stigmasterol and β-Sitosterol were performed using the Lipinski's rule of five and the Veber's rule. According to the Lipinski's rule, a drug must have its molecular weight (MW) ≤ 500, number of hydrogen bond donors (HBD) ≤ 5, octanol / water partition coefficient Log P ≤ 5 and number of hydrogen bond acceptors (HBA) ≤ 10 for it to be orally bio-available. Violation of more than one of these indices could translate to poor drug-likeness potentials of a ligand. Veber's rule, on the other hand indicates that, for a drug to be orally bio-available, the number of rotatable bonds (NRB) must be < 10 and topological polar surface area (TPSA) must be < 140 $Å^2$. The NRB, TPSA, MW, HBD, HBA, and Log P values of the bioactive ligands were computed using the Swiss ADME (www.swissadme.ch/) online tool [34 & 35].

2.6. ADMET Profiling

High failure rates of drug candidates at the late stage of drug development has made prediction of pharmacokinetic and toxicity profiling of therapeutic ligands at the early stage of drug

development important. The template and the designed ligands should be profiled for their gastrointestinal (GI) absorption, blood brain barrier (BBB) permeation, P-glycoprotein (P-gp) substrate potentials, and cytochrome-P450 enzymes inhibition using the Swiss ADME online server at http://www.swissadme.ch/index.php. Furthermore, Osiris DataWarriorV5.5.0 chemo-informatics program was used to perform *in silico* toxicity assay on the selected bioactive ligands using the following toxicity endpoints; mutagenicity, reproductive effect, and irritating effect [34 & 35].

3.0. Results and Discussion

Table 1: Phytochemical screening of the bulb extracts of *Calotropis procera*

Class of Compound	normal-Hexane	Ethyl acetate	Methanol
Saponins	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+
Steroids/Sterols	+	+	+
Alkaloids	+	+	+
Reducing Sugars	+	+	+
Cardiac glycosides	+	+	+
Anthraquinone	_	_	+

Key = + present, - below detectable limits

From the above table, it is indicated that most of the phytochemicals tested for, were present but for anthraquinones that were below detection levels in N. Hexane and ethyl acetate.

3.1. Antimicrobial Activity

Table 2: Sensitivity/zones of inhibitions (mm) of the crude Extract Against microbes

Test organism	**EA**	**MEOH**	**HEXANE**	**Ciprofloxacin**	**Tetracycline**	**Fluconazole**
Nectricillin resistant *Staph. Aureus*	S (24)	S (21)	S (20)	R (0)	S (29)	R (0)
Vancomycin resistant *enterococci*	R (0)	R (0)	R (0)	S (28)	S (30)	R (0)
Staphylococcus aureus	S (27)	S (24)	S (21)	R (0)	S (32)	R (0)
Escherichia coli	S (28)	S (23)	S (20)	S (35)	R (0)	R (0)
Neisseria gonorrhea	S (26)	S (22)	S (18)	R (0)	S (25)	R (0)
Profeus mirabilis	R (0)	R (0)	R (0)	S (32)	R (0)	R(0)
Pseudomonas aeruginosa	R (0)	R (0)	R (0)	S (30)	S (27)	R (0)
Salmonella typhi	S(24)	S (21)	S (18)	S (41)	R (0)	R (0)
Candida albicans	S (25)	S (22)	S (20)	R (0)	R (0)	S (32)

Candida krusei	R (0)	R (0)	R (0)	R (0)	R (0)	S (30)
Candida stellafoidea	S (23)	S (20)	S (17)	R (0)	R (0)	S (34)

Legend =≫ S= Sensitive, R= Resistance, EA= Ethyl acetate extract, MEOH= Methanol extract,

HEXANE= n-hexane extract, Numeric value in brackets = diameter of zone of inhibition in

millimetres; Drug concentration: Ciprofloxacin = 20µg, Tetracychine = 20µg, Fluconazole

= 20µg

Table 2, showed the zones of inhibitions of the various solvents on the microbes. Ethyl acetate has the highest efficacy and efficiency of the applied solvents, (28 mm, with *Escherichia coli*) followed by methanol (24 mm with *Staphylococcus aureus)* and the N. Hexane being the least, (21 mm with *Staphylococcus aureus).*

3.2. Spectroscopic Characterization

^{1}H and ^{13}CNMR spectra of Cp45 were run using $CDCl_3$ as solvent on Agilent-NMR 500MHz spectrophotometer at Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde Glasgow, United Kingdom.

3.3. Characterization of Cp_{45}as a mixture of Stigmasterol and β-Sitosterol

^{1}H NMR spectrum of the compounds varied between 0.736 to 5.34 ppm, This spectrum, (table 3 and fig.2), showed the presence of 6 high intensity peaks portraying the presence of six methyl groups at δ 1.01, 0.86, 0.97, 1.00, 1.27 and 1.534 ppm. The proton corresponding to the H-3 of a sterol moiety was seen to appear as a triplet of doublet at δ 3.52 ppm. At δ 5.13 ppm and at δ 5.34 ppm corresponds to a single peak in the region of the ethylene protons, suggesting the presence of three protons. The proton NMR showed the proton of H-3 appeared as a multiplet at δ 3.52 ppm and revealed the existence of signals for an olefinic proton at δ5.01 (m), 5.13 (m), 5.34 (m), and 2.32(m).

Going by literature, (**Tables3 and 4**), β-sitosterol and Stigmasterol always form a mixture together in greater proportions. Many at times, it is difficult to obtain Stigmasterol in pure state. The only difference between the two is the presence of a double bond between C22 and C23 in Stigmasterol and single bond in β-sitosterol. Further, literatures, (tables 3 & 4), have also shown that β -sitosterol is difficult to be obtained in pure state [36]. Both compounds have the same Rf value of 0.55, thus, stereo isomers. The assignments were coherent in comparison with literature, [37, 36, &38]. Therefore, Cp45 is a mixture of β-sitosterol and Stigmasterol. Studies showed that stigmasterol possess antimicrobial, anti-inflammatory and membrane stabilizing activities [39], whereas sitosterol possesses anti-cancer, antibacterial, antifungal and antidiabetic properties [40].

Table 3: Selected ^{1}H NMR Experimental and Literature data of Stigmasterol

Position	Experimental data of ^{1}H (δ ppm)	[15] Data ^{1}H (δ ppm)	[38] Data ^{1}H (δ ppm)	[36] Data ^{1}H (δ ppm)	[41] Data ^{1}H (δ ppm)
3	3.52	3.25	3.53	3.52	3.51
6	5.13	5.14	5.38	5.36	5.31
18	1.06	1.07	1.29	0.69	0.91

19	1.27	1.26	0.74	1.01	
21	0.91	0.91	1.20	1.02	
22	5.01	4.62	5.07	4.98	5.14
23	4.61	4.61	5.20	5.14	5.14
26	1.02	1.01	0.84	0.75	0.82
27	1.00	1.00	0.97	0.85	0.80
29	0.97	0.97	1.04	0.83	1.03

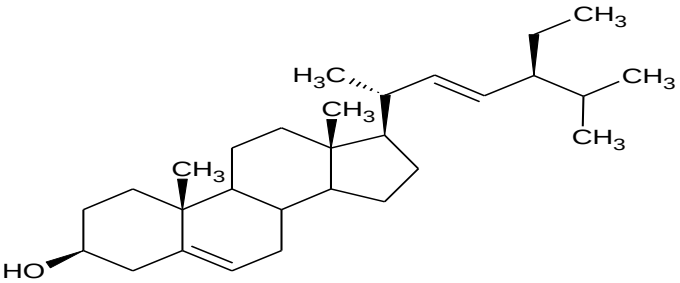


Fig. 2: Stigmasterol

Table 4: Selected ^{1}H NMR Experimental and Literature Data of β – Sitosterol

Position	Experimental Data ^{1}H(δ ppm)	[15] Data ^{1}H(δ ppm)	[38] Data ^{1}H(δ ppm)	[36] Data ^{1}H(δ ppm)	[37] Data ^{1}H δ ppm)	[41] Data ^{1}H(δ ppm)
1	1.15					
2	2.35					
3	3.52	3.25	3.53	3.52	3.52	3.53
4	1.85					
6	5.34	5.31	5.38	5.36	5.36	5.36
18	1.16	1.16	1.29			
19	1.25	1.25	0.74	0.92	0.92	0.93
21	0.91	0.91	1.20	0.91	0.85	
23	1.64					
24	2.35					0.84
26	1.01	1.01		0.83	0.83	0.83
27	0.98	0.98		0.81	0.82	0.81
28	0.86			1.01	1.01	0.68
29	0.96	0.96		0.68	0.68	1.01

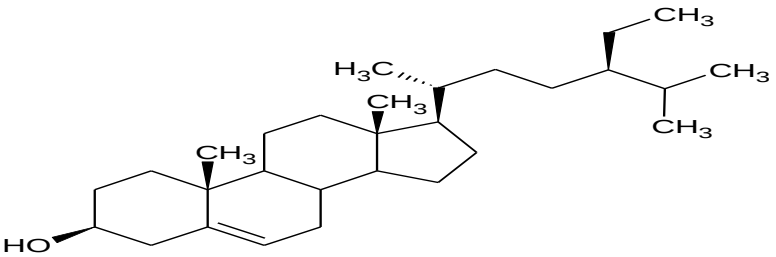


Fig. 3: β – Sitosterol

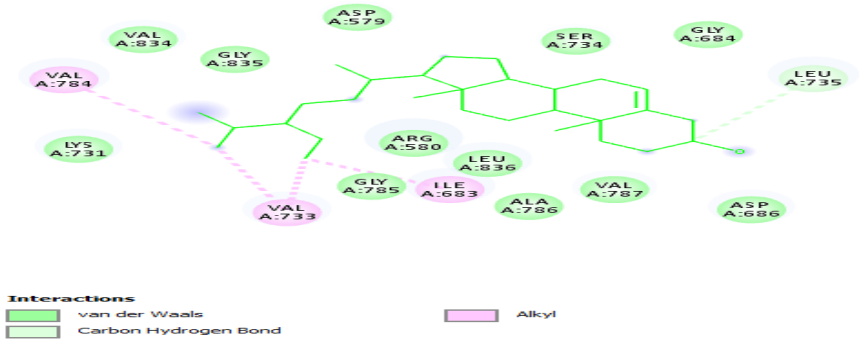
3.5. Molecular Docking Outcome

The mechanism of action of many therapeutic compounds is the inhibition of biochemical and physiological roles of a target macromolecule of a pathogenic microbe binding strongly to the protease, leading to either inactivation or death of the organism. DNA gyrase which is the target macromolecule in the study plays an essential role in the replication process of *Escherichia coli* and *Salmonella typhi*. The protease introduces negative super helical twists into the bacterium's chromosomes and maintains a particular level of super coiling [42]. The results of the molecular docking of Stigmasterol and β-Sitosterol against the active sites of DNA gyrase are presented in Table 5. The bioactive ligands were found to bind to the target protease spontaneously with Gibb's free energy change (ΔG) of -7.8 kcal/mol. When compared with Ciprofloxacin (ΔG = -7.6 kcal/mol), a standard antibiotic used in this study for quality control, the ligands tend to show a 0.2 value higher than the standard.

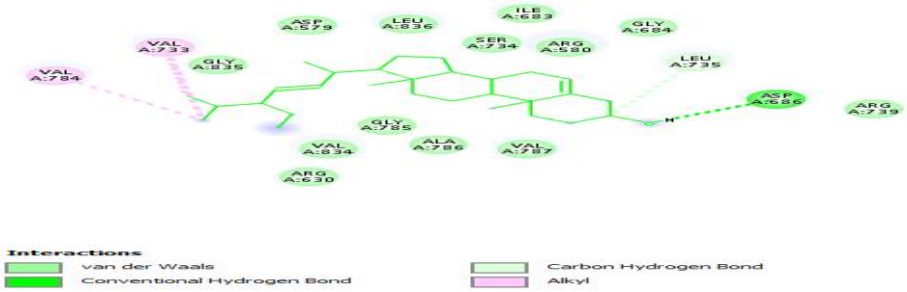
The 2D diagrams of the complexes of the ligands with DNA gyrase are presented in Figures 4. β-Sitosterol formed four alkyl bonds with VAL 784, VAL 733, and ILE 683; and an carbon-hydrogen bond with LEU 735 amino acid side chain of DNA gyrase (Figure 4). Also, Stigmasterol interacted with the amino acid side chains of the target via the formation of two alkyl bonds with VAL 784 and VAL 733; a carbon-hydrogen bond with LEU 735; and a conventional hydrogen bond with ASP 686. Likewise, the reference antibiotic, Ciprofloxacin, by binding to the macromolecule via three alkyl bonds with ARG 630 and a conventional hydrogen bond with ILE 631 tends to inhibit the protease in a mechanism that is totally different from those of the four investigated phytochemicals, (Figure 4C).

Table 5: Binding Affinity of Ligand-macromolecule complex

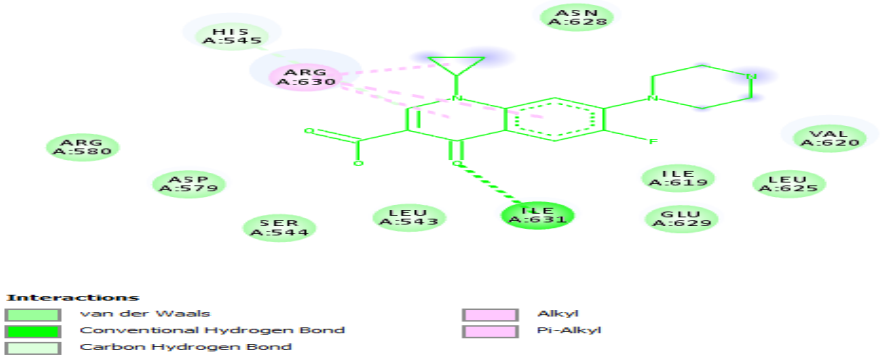
Ligand	ΔG (kcal/mol)
β-Sitosterol	-7.8
Stigmasterol	-7.8
Ciprofloxacin	-7.6



A: Beta-sitosterol/DNA gyrase complex



B: Stigmasterol/DNA gyrase complex



C: Ciprofloxacin/DNA gyrase complex

Fig. 4: Diagram of interaction of the investigated bioactive ligands with the active sites of DNA gyrase

3.6. Oral Bioavailability and ADMET Profiles.

Oral bioavailability / drug-likeness prediction for therapeutic ligands is a fundamental assessment in modern drug discovery and development, owing to the fact that oral delivery remains the most common path of drug conveying, into systemic circulation [35]. This parameter was assessed for the two molecules using the Lipinski's rule of five and the Veber's rule. Results are presented in Table 6, indicating that they obey both rules. Thus, the bioactive compounds could be taken through the oral route.

A major cause of high attrition rate in drug discovery and development is poor pharmacokinetic and toxicity profiles of drug candidates. A way of circumventing this challenge is early prediction of ADMET profiles of drug candidates. Table 7 shows the *in silico* ADMET profile of the investigated molecules. The ligands were found to possess gastrointestinal absorption. The BBB (blood brain barrier) is a layer of endothelial cell that demarcates the brain from blood [43]. The assessment of BBB permeating potentials shown in Table 7 indicated that none of the compounds can penetrate the BBB and as such would not have any influence on the Central Nervous System. Also, P-glyco-proteins (P-gp) are intracellular and extracellular membrane transporters of xenobiotic in the body [44]. It reduces cellular concentrations of its substrates leading to their poor pharmacokinetic profiles. The two investigated molecules were found to be none substrates of P-gp (Table 7). Furthermore, Cytochrome 450 (CYP450) mono-oxygenase is a group of enzymes central to the metabolism and excretion of drugs. The bioactive ligands were screened against five forms of the enzyme. The result (Table 7) revealed that the two molecules are substrate of CYP450 enzyme, indicative of their high probabilities of being bio-transformed and eventually made bioavailable upon oral administration [45]. Another important pharmacokinetic parameter worthy of note especially for therapeutic compounds that requires transdermal administration is the skin permeability (LogKp). The LogKp data of Stigmasterol, and β-Sitosterol presented in Table 7 revealed that the compounds have poor skin penetration potentials due to the negative values of their LogKp [46]. In

addition, the toxicity profiles (Table 7) of the compounds revealed that none of them is mutagenic, tumorigenic, irritating, or pose any negative effects on the reproductive system of the targeted organism.

Table 6: Oral Bioavailability Profiles of the Phytochemicals

Ligand Rule	β-Sitosterol	Stigmasterol
Lipinski's	Yes	Yes
HBA	1	1
HBD	1	1
MW ($gmol^{-1}$)	414.7	412.7
$cLogP_{(o/w)}$	7.2	7.0
Veber's	Yes	Yes
NRB	6	5
TPSA ($Å^2$)	20.23	20.23

HBA; hydrogen bond acceptor, HBD; hydrogen bond donor, Mw; molecular weight, cLogP; consensus octanol water partition coefficient, NRB; number of rotatable bond, TPSA; topological polar surface area

Table 7: *In silico* Pharmacokinetic and Toxicity profiles of the Phytochemicals

Ligand	CYP450 Substrate	GIA	P-gp+	BBB	Mutagenic	Irritant	Reproductive effect	Tumorigenic	LogKp (cm/s)
β-S	Yes	Yes	No	No	None	None	None	None	-2.2
Sg	Yes	Yes	No	No	None	None	None	None	-2.7

GIA; gastrointestinal absorption, BBB; blood brain barrier penetration, $P\text{-}gp^{+}$; P-glycoprotein substrate (β-S; β-Sitosterol, Sg; Stigmasterol).

4.0. Conclusion

The crude extracts of the bulbs of *Calotropis procera* tested for microbial bioassay against eight bacteria and three fungi exhibited various levels of activity against the test microbes. For all the samples, the inhibition effect increases from (0.63mg/mL) to (20mg/mL) compared with the positive controls. The phytochemical screening of the extracts confirmed the presence of most of the phytochemical constituents with potent biological activity from the extracts. Stigmasterol and β-Sitosterol were isolated and purified by column and thin layer chromatographic processes, and characterized by NMR techniques. These compounds portray several bioactive properties viz anti-malarial, anti-ulcer, anti-tumour, antifungal, antibacterial, antidiarrheal, analgesic and other pharmacological properties. The strong activity of the extracts against these microbes *Escherichia coli* and *Salmonella typhi* indicated that these molecules have pharmacological effects. Theoretical studies on their binding interactions with the active sites of DNA gyrase of *Escherichia coli* and *Salmonella typhi* revealed that the compounds bind to the target macromolecule via hydrophobic and hydrogen bond interactions

with binding affinity of -7.8 kcal/mol. In addition, *in silico* drug-likeness and pharmacokinetic ADMET examination of the compounds showed that they obey both Lipinski' and Veber, rules for *in silico* drug likeness.

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