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Isolation, Characterization, Antimicrobial and Theoretical Investigation of Some Bioactive Compounds Obtained from the Bulbs of *Calotropisprocera*

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Abstract

This study characterizes the bioactive molecules from the bulb of *Calotropisprocera* and investigates the antimicrobial activities of the crude extracts. Theoretical studies on the two isolated compounds in the crude extract were also accomplished.The bulbs were air dried, pulverized, and subjected to extraction procedures by maceration using 500 mL each of normal-hexane, ethyl acetate and methanol. The crude extracts were further tested onmicroorganisms and phytochemical screening using standard procedures. In addition, the bioactive compounds in the extract were screened against DNA gyrase of two Gram negative bacterial species; *Escherichia coli* and *Salmonella typhi*using Molecular Docking simulation techniques and further subjected to ADMET profiling,using the Swiss ADME online server. The Crude ethyl acetate extract has 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). β-Amyrin acetate and Taraxasterol are the two phytochemicals in the purified white crystalline fractions and were found to fasten to the active sites of DNA gyrase of the Gram negative bacterial species via hydrophobic and hydrogen bond interactions, with binding activity value of -9.6 kcal/mol and -9.5 kcal/mol, respectively. Also, ADMET investigations of the compounds revealed their sound oral bioavailability and excellent pharmacokinetic and toxicity profiles. The findings of this study could provide a platform for discovering safe and potent antibiotics against pathogenic microbes ravaging our society.

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1. Introduction

Natural products are the most successful precursors of future drug leads [1-5]. It has been, it is, and will continue to

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provide distinctive structural variety that presents opportunities for discovering, mainly new low molecular weight lead molecules [6].

Biosynthesis and a critical analysis of proteins, fatty acids, nucleic acids and carbon derivatives is known as primary metabolism and the molecules are called *primary metabolites* [7]. The mechanism by which an organism biosynthesizes bioactive molecules or *secondary metabolites* is a unique one [8].

These metabolites generally, are not actually needed for growth, development or reproduction of an organism but are produced either for adapting the organism to its surrounding environment or to act possibly for defence mechanism against predators in assisting survival of the organism [9]. Biosynthesis of these metabolites is gained from the fundamental processes of photosynthesis, glycolysis and the Krebs cycle to procure biosynthetic intermediates which, ultimately, results in the formation of these secondary metabolites [7]. Though the number of building blocks is limited, the formation of new secondary metabolites is infinite. Utmost important constructing blocks employed in the biosynthesis of these metabolites are those obtained from the intermediates: Acetyl coenzyme A (Acetyl-CoA), Shikimic acid, Mevalonic acid and 1-Deoxyxylulose-5-phosphate. These are involved in countless biosynthetic pathways, involving numerous different mechanisms, actions reactions, inactions and interactions.

*Calotropisprocera, a*small tree is widely spread in tropical and subtropical Africa [10]. It is called different names in English and locally, like "Sodom apple, Usher, Dead Sea apple, Swallow-wort, Giant milk weed "Tumfafiya or Baabaa ambalee in Hausa, Ewe-Bomubomu in Yoruba, Otokwuru in Igbo and Konzar Atumbagh in Tiv [11]. 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 produce large quantities of latex when cut or broken [12]. *Calotropisprocera (Asclepiadaceae)* is recognised by most traditional systems of medicine as 'Madar" in Unani medicinal system. Earliest Hindu writers mentioned the plant and its primeval name occurred in the Vedic literature was "Arka" alluding to the form of leaves, which was used in the sacrificial cremation described by the Sanskrit writers [13].

The Latex exuded by *Calotropisprocera* is valued for its high medicinal and pharmaceutical activity due to its high content of bioactive compounds such as;- cardiac glycosides, alkaloids, terpenes, resins, lipids, flavonoids, tannins and steroids [14]. This latex possesses different biological activities including: anti-inflammatory, analgesic, antitumor, antiviral, hepatoprotective, antiulcer, anthelmintic, insecticidal, herbicidal, antioxidant and spasmolytic activities [15].

Theoretical 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 [16-18]. 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 base of bioactive ligands [20-25, 18]. Likewise, pharmacokinetic investigation which is concerned with the fate of therapeutic ligands in the biological system forms an essential component of modern drug discovery. This deals with the absorption, distribution, metabolism, excretion, and toxicity (i.e., 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 [26-28].This study is aimed at isolation, characterization and application of *in vitro* & *in silico* techniques to explore the bioactive molecules present in Bulbs of *Calotropisprocera*.

2. Methods

2.1. Sample Collection

The fresh bulbs of the plant identified as *Calotropisprocera* (Figure 1) was collected from Millionaires' Quarters, Lafia L. G A. of Nasarawa State Nigeria, in August, 2019 and was 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. The bulbs were washed with clean water to removed dirt and air dried at room temperature. The dried fruits were then pulverized into coarse powder with mortar and pestle, then sieved with a sieve of 0.55 mm pores and stored in cellophane bags at room temperature until required for experimental use.

Figure 1: Plant and bulbs of *Calotropisprocera*

2.2. Extraction

500g of the plant material was weighed and extracted by the process of Maceration, allowing the pulverized powdered material to soak in an appropriate solvent in a closed vessel at room temperature. Three chosen solvents were employed, in the following order, normal-Hexane, Ethyl Acetate and Methanol. The plant sample was macerated with two litres of each of the solvents for 72 hours with agitation. The above was filtered and the filtrate concentrated under reduced atmospheric pressure using a rotary evaporator at 37^{O}C, to recover some of the

solvent. This procedure was repeated for the ethyl acetate and methanol extracts. They were then allowed to dry. The various solvents extracts were coded for quick identification during further analysis.

2.3. Screening for Phytochemicals

Crude extracts from the used solvents were phytochemically evaluated for the presence of anthraquinones, saponins, tannins, steroids, terpenes, reducing sugars, flavonoids and alkaloids using standard scrutiny as reported by [29, 30]. Phytochemical screening results showed the presence of saponins, tannins, steroids/sterols, tannins, terpenoids, flavonoids, reducing Sugars, and cardiac glycosides.

2.4. Bioassay

Antibacterial and antifungal activities of the normal- 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, Profeusmirabili, Pseudomonas aeruginosa, Salmonella typhi, Candida albicans, Candidakrusei* and *Candida stellafoidea.* Dispersion method was used for screening the extracts. Mueller Hinton Agar was used as the growth medium for microorganisms, sterilized at 121°C for 15 min, poured into Sterile Petri Dishes and were cooled and solidified. The said crude extracts (0.4 g) were allowed to dissolve in 10.0 mL of Dimethylsulphoxide (DMSO) to acquire a concentration of 40.0 mg/mL. A disinfected medium was then seeded with the standard Inoculum (0.1 mL) of test microorganisms and were equitably spread 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 5.0 mg/mL of the already weighed crude extract was then launched 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 [31]. Minimum inhibition concentration (MIC) of these extracts were carried out using Broth Agar Dilution method. Mueller Hinton Broth was prepared by dispensing 10.0 mL into test tubes and pasteurised at 37°C for 6 hours, then cooled. McFarland's turbidity standard scale number 0.5 was prepared to give a turbid solution. Normal Saline (10.0 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 tubes were observed for turbidity. The lowest concentration of extracts in the sterile broth that indicated no turbidity was recorded as the minimum inhibition concentration (MIC) [32]. The minimum bactericidal and minimum fungicidal concentration (MBC and MFC) were carried out to determine the concentration of extracts that could stop growth of tested microorganisms. Mueller Hinton Agar was prepared, pasteurised at 121^{O}C within 15 minutes, poured into sterile Petri dishes and allowed to cool. The contents of the test tubes with the evaluated MIC were then sub-cultured onto prepared media, incubated at 37^{O}C for 24 hrs, after which the plates were visualised for any colony growth. MBC/MFC plates with lowest concentration of extract, without a colony growth were considered as the MBC/MFC [32].

2.5. Spectroscopic Characterization

^{1}H and ^{13}CNMR spectra of β -Amyrin acetate (Cp_{17}) and Taraxasterol (Cp_{35}) 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.

2.6. Molecular Optimization and Docking Procedures

Antimicrobial studies on the crude extracts revealed that they possess profound bioactivities against *Escherichia coli* and *Salmonella typhi*. Also, phytochemical screening divulged the presence of β -Amyrin acetate and Taraxasterol. Guided by these findings, the two bioactive 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 gyraseof *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 calculation 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 X: -26.4401 Å, Y: 23.0598 Åand Z: 22.0813 Åwith dimensions of X: 51.6292 Å, Y: 53.0044 Åand Z: 51.8136 Å[26-27, 33-34].

2.7. 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 β -Amyrin acetate and Taraxastero 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) $\leq$ 500, number of hydrogen bond donors (HBD) $\leq$ 5, octanol/ water partition coefficient Log P $\leq$ 5 and number of hydrogen bond acceptors (HBA) $\leq$ 10 for it to be orally bioavailable and 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 stipulates that, for a drug to be orally bioavailable, 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 value of the bioactive ligands were computed using the Swiss ADME (`http://www.swissadme.ch/`www.swissadme.ch/) online tool [26-27, 33-34].

2.8. ADMET Profiling

The high failure rates of drug candidates at the late stage of drug development has made prediction of pharmacokinetic and toxicity profiles of therapeutic ligands at the early stage of drug development a necessity. The phytochemicals were 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 Data Warrior V5.5.0 chemoinformatics program was used to perform *in silico* toxicity assay on the ligands using the following toxicity endpoints; mutagenicity, reproductive effect, and irritating effect [26-27, 33-34].

3. Results

3.1. Phytochemical and Antimicrobial Screening

Phytochemical and antimicrobial screening of the crude extract results are presented in Tables 1 and 2, respectively.

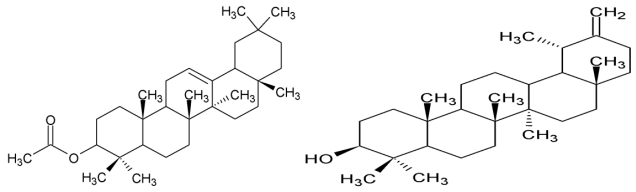
Table 1: Phytochemical screening of the bulb extacts of *Calotropisprocera*

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

Key = (+) indicate, present, (-) indicate, below detectable limits

3.2. Elucidated Structures of the Bioactive Compounds

Figure 2 presents elucidated structures of the two bioactive ligands present in the crude extract of the Bulbs of *Calotropisprocera*. Experimental and literature data for ^{1}H and $^{13}CNMR$ analysis are presented in Tables S1-S4 in the supplementary file (below).

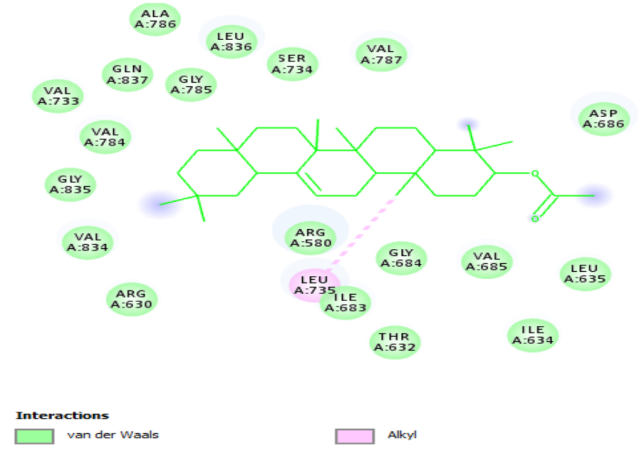


A :β- Amyrin acetate B: Taraxasterol

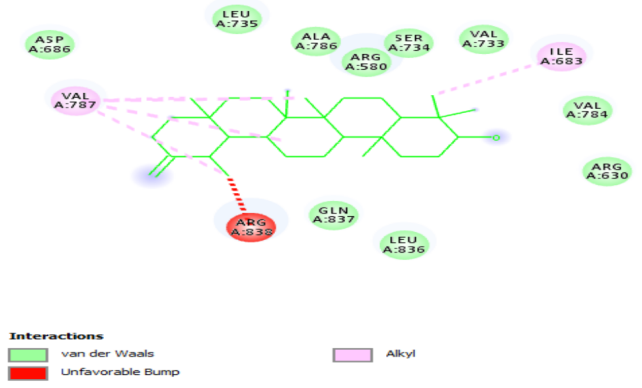
Figure 2: Elucidated Chemical Structures of Bioactive compounds in the Extract

3.3. Molecular Docking Outcome

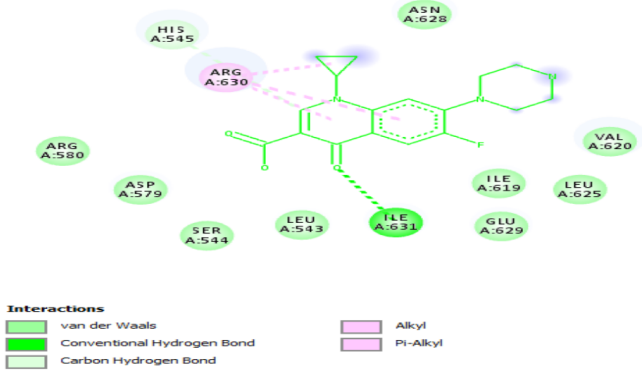
The molecular docking simulation derived binding affinities and diagrams of interaction of the ligands with the active sites of the DNA gyrasetarget are presented in Figure 3.



A: Beta-amyrin-acetate/DNA gyrase complex (ΔG = -9.6 kcal/mol)



B: Taraxasterol/DNA gyrase complex (-9.5 kcal/mol)



C: Ciprofloxacin/DNA gyrase complex (-7.6 kcal/mol)

Figure 3: Binding affinity and Diagrams of interaction of the investigated bioactive ligands with the active sites of DNA gyrase

4. Oral Bioavailability and ADMET Profiles of the Phytochemicals

Oral bioavailability of a therapeutic ligand is a function of some of its physicochemical properties. Tables 3 and 4 present

Table 2: Sensitivity/zone of inhibition (mm) of the crude Extracts Against microorganisms

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 = 20 μ g, Tetracychine = 20 μg, Fluconazole = 20 μg

the descriptors of oral bioavailability and ADMET profiles of the ligands, respectively.

Table 3: Oral Bioavailability Profiles of the Phytochemicals

Ligand Rule	β-Amyrin acetate	Taraxasterol
Lipinski's	Yes	Yes
HBA	2	1
HBD	0	1
MW ($gmol^{-1}$)	440.7	426.7
$cLogP_{(o/w)}$	7.0	7.1
Veber's	Yes	Yes
NRB	2	0
TPSA ($Å^2$)	26.3	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

5. Discussion

Phytochemical screening of the crude extracts of the plant divulged the presence of reducing sugars, saponins, steroids, tannis, alkaloids and flavanoids. Anthraquinones were below detection level (Table 1). This is corroborated with the work of (35), when they analysed the root bark of *Terminaliaschimperiana*.

Screening for Antimicrobials 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.* Parameters determined were the zone of inhibition (ZI), minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC). The antibacterial and antifugal results obtained showed that ethyl acetate extract had the highest diameter of zones of inhibition (28 mm) against (35 mm) for the tested microbes. Over all, ethyl acetate has the highest efficacy and efficiency of the applied solvents, followed by methanol and the N. Hexane. Out of the eleven (11) microorganisms employed, seven (7) had various degrees of activities, with the exception of, *Vancomycin* resistant *enterococci, Profeus mirabilis, Pseudomonas aeruginosa* , and

Table 4: 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 -Ac	Yes	Yes	No	No	None	None	None	None	-2.6
Ta	Yes	Yes	No	No	None	None	None	None	-2.4

GIA; gastrointestinal absorption, BBB; blood brain barrier penetration, P-gp$^+$; P-glycoprotein substrate β-Ac; β -Amyrin acetate, Ta; Taraxasterol

Candida krusei, all of which had zero activities, as seen in Table 2.

The proton analysis (^{1}H NMR spectra) of β -Amyrin acetate (Table S2), showed the presence of the trademark amyrinolefinic proton (^{1}H-12) resonating as triplet at δ 5.16 ppm due to deshielding caused by the π bond. The proton NMR signals at δ 0.85, 0.98, 0.94, 0.81, 1.11, 0.85, 0.77, and 0.92 ppm depict eight methyl groups all singlets with an additional methyl group at δ 2.02 being the acetate methyl protons. At δ 4.47ppm, the double doublet is due to the lone proton on C-3 bonded equatorial to an acetate ester. Broad singlet at δ 0.85 ppm of C-29 and C-30 methyl protons is a pointer to the characteristics of β – amyrin. Furthermore, ^{13}C NMR of the lone proton δ 4.47 (Table S1), is assigned H-3 by HSQC correlation with δ 80.35 and HMBC- *3J* correlation to methyls δ 15.71 (C-23), δ 28.30 (C-24) and δ 170.94 ppm (C-1') from the acetyl moiety. HSQC correlation of δ 21.53 to δ 2.02 (3H) is evident of electron rich δ 170.94 ppm (C-1') of the acetyl moiety. The acetate is attached to the first hexacyclic component at C-3. δ 5.16 and correlates with δ 121.68 ppm on HSQC and is ascribed H-12. It shows a HMBC- *2J* correlation with the secondary carbon δ 23.75 (C-11) and *3J* quaternary carbon δ 42.07ppm (C-14). H-12 bonds to the electron rich alkenyl carbon (C-12) and splitting with δ 1.80 (H-11) thus, resolved as triplets. δ 33.42 (C-29) axial and δ 23.86 ppm (C-30) equatorial correlations to ^{1}H-19a, 1H-19b and 3H-28 indicates their association to ring E of amyrin. Moreover, δ 0.85 (C-30 & C-29) *2J* correlation to the quaternary δ 31.10 ppm (C-20) indicates the β – functionality of these methyls. The compound / molecule was established as β – Amyrin Acetate (Figure 2A), on the basis of spectral data (1D^{1}H-NMR, ^{13}C NMR, 2D HSQC, HMBC spectroscopy, melting point 242^{O}C, and TLC. More so, comparison with literature data (36-37), confirmed the molecule as β –Amyrin Acetate. Studies show this compound to possess profound anti-inflammatory, anti-malarial and anti-rheumatism activities (38) and (39).

Spectroscopic signals in the PMR (Table S4) and ^{13}C NMR (Table S3) spectra of Taraxasterol were assigned completely using one- and two-dimensional (2D) NMR methods (HSQC and HMBC). The weak-field region of the PMR of the compound contains signals for three protons. Based on cross-peaks in the HSQC, the first two protons at 5.10 ppm belong to C-30 with Chemical shift 107.25 ppm. The doublet of doublets at 4.70 ppm corresponds with the proton on C-3 (80.75 ppm) of ring A. Cross-peaks with C-2 and a quaternary C atom at 37.74 ppm (C-4) are found in the HMBC spectrum for H-3. Cross-peaks with protons at 0.78 ppm (^{1}H, triplet; corresponds with the C atom with Chemical Shift (CS) 55.42 ppm) at HSQC. The protons with Chemical shift 0.91 ppm in the HSQC correspond with signals for C atoms with Chemical Shifts 28.06 and 16.36 ppm of the *gem*-dimethyls C-23 and C-24. Their protons also correlate with tertiary (55.42 ppm) and quaternary (37.74 ppm) C atoms C-5and C-4, respectively. Their characteristic atoms C-6 (18.37 ppm) and C-7 (34.13 ppm) are found using the HSQC spectra. Protons H-1 and H-5 and protons of the methyl with Chemical Shift 0.86 ppm, give correlations in the HMBC spectrum with a quaternary C atom with δ 38.01 ppm. These are C-10 and methyl C-25. In the HSQC spectrum, the last signal correlates with H-9 with δ1.33 ppm which, in turn, correlates with C-10 and yet another quaternary C atom with Chemical Shift 40.62 ppm (C-8), C-8 couples with methyl protons at 0.99 ppm (on C-26 with δ16.36 ppm). The doublet for methyl protons H-29 at 1.08 ppm in the HSQC spectrum corresponds with the C atom at 25.60 ppm(C-29). Corresponding cross-peaks in the HMBC spectrum between methyl protons in ring D at 1.08 ppm and C atoms at 38.30 (C-19) and 154.65 ppm (C-20) in addition to C-29 at 25.60 ppm and a proton at 2.15 ppm (H-19) confirm that the assignments were correct. Methyl protons with CS 0.94 ppm, which correspond in the HSQC spectrum with the C atom at 19.58 ppm (C-28) have correlations in the HMBC spectrum with C atoms at 34.41 and 48.70 ppm (Tables S3 and S4). These assignments in comparison with literature data (40-41), confirmed the compound to be taraxasterol (Figure 2B). The presence of carbon spectrum in 145ppm and 178ppm at the ^{13}C NMR indicate the presence of fatty acid impurities. This compound was reported to have many biological properties, including anti-inflammatory and anti-tumour activities [42].

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 [34]. This essential parameter was assessed for the two phytochemicals taking cognisance of Lipinski's rule of five and the Veber's rule. The results, thus, presented in Table 3, implies that they obey both rules. Thus, the extract 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 4 presents the *in silico* ADMET profile of the two investigated

phytochemicals. All the ligands were found to possess good gastrointestinal absorption. The blood brain barrier (BBB) is a layer of endothelial cell that demarcate the brain from blood [44]. The assessment of BBB permeating potentials of the compounds shown in Table 4 portrayed that none of the compounds can penetrate the BBB and as such would not have any influence on the Central Nervous System.

Also, P-glycoproteins (P-gp) are intracellular and extracellular membrane transporters of xenobiotic in the body [45]. It reduces cellular concentrations of its substrates leading to their poor pharmacokinetic profiles. All the investigated phytochemicals were found to be none substrates of P-gp (Table 4).

Furthermore, Cytochrome 450 (CYP450) monooxygenase is a group of enzymes central to the metabolism and excretion of drugs. The bioactive ligands were screened against five isoforms of the enzyme. The result (Table 4) revealed that all the phytochemicals are substrate of CYP450 enzyme indicative of their high probabilities of been bio-transformed and eventually made bio available upon oral administration [46].

Another important pharmacokinetic parameter worthy of consideration especially for therapeutic compounds that requires transdermal administration is the skin permeability (LogKp). The LogKp data of β -Amyrin acetateand Taraxasterol presented in Table 4 revealed that both the compounds have poor skin penetration potentials due to the negative values of their LogKp (45). Additionally, the toxicity profiles (Table 4) of the compounds revealed that none of them is mutagenic, tumorigenic, irritating, or pose any adverse effect on the reproductive system.

6. Conclusions

Crude extracts of the bulbs of *Calotropisprocera*were investigated for the antimicrobial activities against eight bacteria and three fungi species. The extracts were found to exhibit significant inhibitory activities against the microbes with the crude ethyl acetate extract having 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). The phytochemical screening of the extracts confirmed the presence of β -Amyrin acetate and Taraxasterol. Theoretical studies on the binding interaction of the two identified phytochemicals 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 -9.6 kcal/mol and -9.5 kcal/mol for β -Amyrin acetate and Taraxasterol, respectively. The phytochemicals were found to be better inhibitors of DNA gyrase when compared with the standard Ciprofloxacin ligand which binds to the target macromolecule with binding affinity of -7.6 kcal/mol. In addition, *in silico* drug-likeness and ADMET investigations on the compounds showed that they obey both the Lipinski's rule of five and the Veber's rule in addition to displaying excellent pharmacokinetic and toxicity profiles.

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Appendix

Supplementary file:

Table S1: ^{13}C NMR Experimental and Literature Data of β-Amyrin acetate

Position	Experimental data ^{13}C (δppm)	Okoye*et al.,* 2014 ^{13}C (δ ppm)	Ushie*et al.,* 2018 ^{13}C (δ *ppm*)	Chaturved-ula&rakash, 2013 ^{13}C (δ *ppm*)	Abdullahi*et al.,* 2017 ^{13}C (δ *ppm*)	Wau, 2016 ^{13}C (δ *ppm*)	Ercil*et al.,* 2004 ^{13}C (δ *ppm*)
1	38.91	38.79	38.70	39.30	38.60	38.40	38.14
2	26.70	27.44	28.80	28.30	23.90	23.70	27.51
3	80.35	79.24	79.10	79.60	80.60	81.10	79.12
4	38.91	38.99	38.5	39.10	38.60	37.80	38.88
5	55.40	55.37	55.5	55.10	51.50	55.30	55.27
6	18.24	18.58	18.80	18.80	18.20	18.70	18.44
7	32.85	32.85	33.10	33.20	33.30	32.80	33.03
8	40.05	40.21	38.80	39.80	37.80	39.90	38.82
9	47.59	47.43	49.20	48.00	43.30	47.40	47.81
10	37.08	37.15	36.70	37.40	36.80	36.80	37.00
11	23.75	23.75	22.70	23.90	22.70	23.70	23.48
12	121.68	121.93	116.80	122.20	129.80	122.30	121.82
13	145.21	145.41	142.70	145.70	142.70	145.50	145.28
14	42.07	41.92	41.30	42.10	42.80	41.60	42.18
15	26.36	26.36	27.40	26.00	27.90	27.00	26.12
16	26.80	27.14	27.10	26.30	26.20	26.20	27.37
17	32.70	32.70	49.20	32.60	32.50	32.60	32.04
18	48.04	47.84	33.30	48.10	55.60	47.50	47.22
19	46.85	47.03	48.70	47.30	40.80	46.99	46.93
20	31.10	31.30	29.10	31.30	41.70	31.30	31.34
21	38.20	37.35	35.10	34.30	31.30	34.80	34.83
22	34.56	34.94	37.50	37.40	42.80	37.20	37.26
23	15.71	15.71	15.43	16.10	16.70	16.85	28.22
24	28.30	28.31	29.70	28.20	29.70	28.40	15.47
25	16.01	15.80	15.40	16.10	16.60	16.00	15.72
26	16.92	17.01	17.54	17.20	16.10	17.00	16.97
27	26.21	26.62	21.32	24.40	18.10	26.00	25.56
28	28.60	28.62	28.00	28.20	27.90	28.80	28.44
29	33.42	33.56	33.70	33.90	27.50	33.40	33.44
30	23.86	23.91	25.90	23.80	25.30	23.60	23.48
1[1]	170.94	171.53	170.91	171.00	173.71	171.10	171.01
2[1]	21.53	21.53	29.80	21.53	21.20	21.30	21.80

Table S2: ^{1}H Experimental and Literature Data NMR of $\beta-$ Amyrin acetate

Position	Experimental data ^{1}H (δ ppm)	Okoye*et al.,* 2014 ^{1}H (δ ppm)	Ushie*et al.,* 2018 ^{1}H (δ ppm)	Abdullahi*et al.,* 2017 ^{1}H (δ ppm)	Ercil*et al.,* 2004 ^{1}H (δ ppm)	Chaturvedula and Prakash, 2013 ^{1}H (δ ppm)	Wau, 2016 ^{1}H (δ ppm)
1	1.61	1.49	1.31				1.65
2	1.64	1.55	1.67	1.60			1.64
3	4.47	3.20	3.20	4.47	3.23	3.26	4.50
4	-	-	-	-	-		-
5	0.81	0.71	0.86	0.81	0.87	0.69	0.87
6	1.60	1.53	1.58	1.57			1.54
7	1.52		1.28				1.36
8	-	-	-	-	-	-	-
9	1.63	1.95	1.65	1.58			1.58
10	-	-	-	-		-	-
11	1.80	1.84	1.96	2.25			1.86
12	5.16	5.16	5.50	4.83	5.12	5.18	5.18
13	-	-	-	-			-
14	-	-	-	-			-
15	2.00		1.99				2.00
16	0.79		1.60				0.97
17	-	-	-				-
18	2.28	1.89	2.04	2.23			1.97
19	1.63	1.59	1.93	2.24			1.67
20	-	-	-	2.27			
21	1.72	1.66	1.31		1.13		1.38
22	0.88		1.63	-			1.39
23	0.77	0.77	0.82	0.90	0.83	0.77	0.86
24	0.98	0.98	0.84	0.84	0.84	0.91	0.87
25	0.92	0.92	0.93	0.91	0.94	0.94	0.97
26	0.94	0.94	0.95	0.98	0.79	0.76	0.96
27	1.11	1.11	0.97	1.04	0.97	1.21	1.13
28	0.81	0.81	1.00	0.83	0.99	1.09	0.83
29	0.85	0.85	1.10	0.86		0.85	0.87
30	0.85	0.85	1.20	0.87		0.78	0.87
1^1	-	-	-	-		-	-
2^1	2.02	2.01	2.07	1.57		2.06	2.05

Table S3: The ^{13}C NMR Experimental and Literature Data of Taraxasterol

position	Experimental data ^{13}C (δ *ppm*)	Khalilov*et al.,* 2003 ^{13}C (δ *ppm*)	Reynold*et al.,* 1985 ^{13}C (δ *ppm*)	Alavi and Yekta, 2008 ^{13}C (δ *ppm*)	Shakurova *et al.,* 2008 ^{13}C (δ *ppm*)	Mahato and Kundu, 1994 ^{13}C (δ *ppm*)
1	38.45	38.40	38.44	38.90		38.80
2	23.60	23.60	23.70	27.70		23.70
3	80.75	80.80	80.96	79.00		79.00
4	37.74	37.70	37.79	38.70	80.94	38.80
5	55.42	55.40	55.40	55.20		55.40
6	18.37	18.10	18.18	18.40		18.30
7	34.13	33.90	33.99	34.00		34.10
8	40.62	40.80	40.91	40.80		40.90
9	50.47	50.30	50.39	50.40		50.50
10	37.00	37.00	37.04	37.00		37.10
11	21.50	21.40	21.46	21.50		21.50
12	25.52	25.50	26.15	26.10		26.20
13	38.80	38.80	39.15	39.20		39.20
14	41.85	41.90	42.03	42.10		42.00
15	26.60	26.60	26.64	26.50		26.70
16	39.00	39.10	38.29	38.40		38.30
17	34.41	34.40	34.53	34.40		34.50
18	48.70	48.60	48.63	48.70		48.70
19	38.30	38.30	39.28	39.40		39.40
20	154.65	154.40	154.64	154.60	154.54	154.70
21	25.62	25.40	25.61	25.50		25.60
22	38.93	39.30	38.85	38.90		38.90
23	28.06	27.80	27.94	28.00	28.78	28.00
24	16.36	16.40	16.51	15.40	16.48	15.40
25	15.51	15.40	16.34	16.90	14.54	16.80
26	16.36	16.20	15.89	16.00	16.11	15.90
27	14.85	14.60	14.72	14.90	14.09	14.80
28	19.58	26.10	19.49	19.40	19.53	19.50
29	25.06	19.40	25.49	25.50	25.94	25.50
30	107.25	107.00	107.12	107.20	107.23	107.10

Table S4: ^{1}H NMR Experimental and Literature Data of Taraxasterol

Postion	Experimental data ^{1}H (δ *ppm*)	Khalilov*et al.,* 2003 ^{1}H (δ *ppm*)	Alavi and Yekta, 2008 ^{1}H (δ *ppm*)	Mahoto and Kundu, 1994 ^{1}H (δ *ppm*)
1	0.92	0.92		
2	1.67	1.67		
3	4.70	4.70	3.22	3.22
4	-	-		
5	0.78	0.78	0.77	
6	1.52	1.46		
7	1.35	1.35		
8	-	-		
9	1.33	1.33		
10	-	-		
11	1.51	1.48		
12	1.65	1.65	1.03	
13	1.56	1.56		
14	-	-		
15	1.65	1.65	0.93	
16	1.17	1.26		
17	-	-		
18	0.99	0.99		
19	2.15	2.15		
20	-	-		
21	2.48	2.48		
22	1.41	1.41		
23	0.91	0.91	0.77	0.71
24	0.91	0.91	0.85	0.77
25	0.86	0.86	0.86	0.86
26	0.99	0.99	1.02	0.97
27	0.96	0.96	0.93	
28	0.94	0.94	0.85	0.98
29	1.08	1.08	1.02	0.96
30	5.10	4.79	4.62	4.66