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The attenuation of doxorubicin-induced testicular toxicity with improved testicular histoarchitecture of mice by the bioactive compounds in *Solanum anomalum* leaves: Experimental and computational studies

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ABSTRACT

Doxorubicin, as an antibiotic causes toxicity in human tissues through the generation of oxidant species; however, *Solanum anomalum* (Solanaceae) is ethnopharmacologically and scientifically reported to possess antidotal activities. This study was designed to validate the antidotal potency of the plant's bioactive compounds on rats' testes following induction with doxorubicin through the evaluation of oxidative stress markers, lipid peroxidation indices, testes' histological sections, and *in silico* profiling of the plant's bioactive compounds against some proteins. The collection and preparation of the plant extract, testicular toxicity induction, seminal analysis, assay of testosterone and oxidative stress markers, lipid peroxidation profiling, histomorphological studies, retrieval of catalase, superoxide dismutase, and glutathione peroxidase from PDB, GC-MS, ADME, and docking analyses followed standard protocols. In addition, Swiss-ADME and Auto Dock Vina 4.2 tool enabled drug-likeness, pharmacokinetic properties, and molecular docking analyses. The administration of differential dosages (70–210 mg/kg) of the extract to male rats induced with doxorubicin revealed that the serum levels of malondialdehyde (MDA), total cholesterol (TC), triglycerides (TG), LDL-C, and VLDL-C were significantly decreased, whereas significant increases were observed in the levels of HDL-C, testosterone, GSH, SOD, GPx, and CAT when compared to negative control animals. The histological findings suggested strong testicular protective potential that corroborated the chemical pathological alterations. Therefore, the compounds (squalene, β-sitosterol, cispinane, 1,4-Eicosadiene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, heptacosane, and bicyclo-heptanes-2,5,6-trimethylsilyl) characterized from *S. anomalum* leaf that revealed remarkable binding energies, pharmacokinetics, physicochemical, and drug-likeness properties contributed to the attenuation of the doxorubicin-induced testicular toxicity; hence, they possess antidotal activities.

1. Introduction

Doxorubicin (DOX) is an anthracycline glycoside antibiotic that possesses potent activity against human tissues due to its metabolite (DOX-semi-quinone), an unstable metabolite that reacts with O_2 to generate various free radical species. According to Arunachalam et al. [1] and Tirupathi-Pichiah et al. [2], DOX inhibited adipogenesis via the lowering of PPAR expression, leading to the prevention of spermatogenesis; thereby, resulting in the epididymal adipose tissue abnormalities, an implication for healthy spermatogenesis. Other studies have demonstrated that it could impair sperm motility, increase the percentage of abnormal spermatozoa, decrease body weight, testicular weight, testosterone levels, and ultimately could lead to testicular failure through oxidative stress and cell apoptosis in testicular tissues [3]. In

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meiotically dividing spermatocytes and spermatogonia, DOX metabolites intercalated into deoxyribonucleic acid (DNA) strands and caused double-strand DNA breaks and cell death [4]. It could induce oxidative stress and death of germ cells, affecting both steroidogenesis and spermatogenesis, thereby resulting in male infertility [5,6]. Moreover, the testicular integrity is negatively impacted by DOX exposure during both the pre-pubertal and post-pubertal developmental phases; hence, the number of germ cells significantly decreased at concentrations that are comparable to human therapeutic dosages [7]. Additionally, a report has shown that the exposure to prepubertal DOX resulted in a long-term germ cell DNA damage and the termination of testicular development [8].

Consequently, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are endogenous antioxidant proteins that play key roles in the body's defense against reactive oxygen species (ROS) generated through DOX induction and ROS could destroy cells and advance a number of diseases [9]. However, some pathologies need the mediation of exogenous antioxidant compounds from some medicinal plants to avert the pathogenesis induced by ROS. An example of a plant with these antioxidant compounds is *Solanum anomalum (S. anomalum)*. Conversely, Nigeria is a country with many tropical rainforests and diverse species of medicinal plants including *S. anomalum*, a plant whose fruits and leaves have both pharmaceutical and nutritional uses [12]. According to Offor and Ubengama [10], the plant's parts are used by traditional medical practitioners for the treatment of diabetes, inflammation, and discomfort. The fruits and leaves have been shown to have hypoglycemic and anti-diabetic properties [11]. More specifically, the leaf extract has been reported to have the following activities: antioxidant, antiplasmodial, anti-oedema, analgesic, and antidiarrheal [12], and D-glucopyranoside, uracil, 5-methyluracil, 1-octacosanol, and octacosane have been characterized from it [12].

Moreover, some of the challenges in the integration of traditional medicine in conventional medical practices include but not limited to the understanding of the plants' chemical constituents as well as their mechanism of actions. The earlier could be ascertained through chromatographic and spectroscopic techniques while the later, through *in silico* studies such as molecular docking [13]. Molecular docking has aided ligand-protein interactions, as well as the discovery and development of new chemotherapies. So, the goal of ligand-protein docking is to predict the predominant binding mode of a ligand with a protein; thus, the understanding of the molecular interactions between potential drugs and their target proteins could enhance the treatment of doxorubicin-induced testicular toxicity. However, as a result of the numerous medicinal assertions for the plant leaf, this study was carried out to validate the antidotal activities of the plant through oxidative stress markers and lipid profiling, seminal analyses, histological studies on the testicular tissues, as well as the isolation and characterization of the constituents in the plant leaf. Docking studies was also carried out for the first time on these compounds to unravel their structural and binding affinities for SOD, CAT, and GPx proteins in order to accentuate the antioxidant and antioxidative stress lead compounds with favorable pharmacological characteristics for lead optimization.

2. Materials and methods

2.1. Plants collection

In August 2022, fresh leaves of *S. anomalum* were collected from compounds in the Uruan area, Akwa Ibom State, Nigeria. A plant taxonomist identified, authenticated the plant, and voucher specimen UUH.75a was deposited in the Department of Pharmacognosy and Natural Medicine herbarium, University of Uyo, Nigeria.

2.2. Extraction

S. anomalum fresh leaves were cleaned, washed, cut into smaller pieces, and allowed to dry for two weeks in a shade. With the aid of an electric blender, the leaves were further ground into powder (1.5 kg), and the powdered leaves were macerated in 7.5 L of 70 % ethanol for 72 hours at room temperature (26 °C). Following filtration with sieve cloth and filter papers, the filtrate was concentrated and dried in a rotary evaporator (40 °C) (BuchiLab, Switzerland). The obtained extract (496.5 g with % yield 33.1 %) was kept in a refrigerator (-4°C) and employed for the study [20].

2.3. Animals

Male Albino Wistar rats (120–135 g) procured from the animal house of the department of pharmacology and toxicology at Madonna University in Elele, Nigeria, were used for the research. The animals were kept in typical cages and fed with typical pelleted diet (Guinea feed) and water. The ARRIVE recommendations were judiciously adhered to, as well as the NIH protocols for handling, use, and care of experimental animals (NIH publication No. 8023, amended 1978). The animal ethics committee of the department gave its approval (approval number: MUEPHARM/0126) and made sure the experimental protocol was strictly followed.

2.4. 2.4 Acute toxicity determination

Using the method described by Lorke 1983 and slightly modified by Asanga et al. [37], the acute toxicity test of the ethanol extract of *S. anomalum* leaf administered to mice was evaluated with sixteen mice in both phases I and II. Lethal dose (LD_{50}) and effective doses (ED_{50}) were geometrically calculated after observing the mice for 24 hours, while the number of deaths were recorded in order to establish the medial dosage of the plant as well as the one that might induce toxicity in a short term in mice. After administering the ethanol extract (50–2000 mg/kg) through the peritoneum, toxicity symptoms such as gasping, slowed breathing, palpitations, and death were seen within 24 hours.

2.5. Experimental design

The Olorundare et al. [14] repeated dosage model, which had a 14-day duration, was used in this study. Rats in Group I received an oral pretreatment of 10 ml/kg/day of distilled water and served as the normal control group. DOX (®Celondoxily Injection 50, CELON Laboratories PVT. Limited, India) dissolved in 0.9 % normal saline was given to group 2 rats for 14 days in addition to normal saline (10 ml/kg/day) as the negative control group. Rats in groups 3–5 received 2.5 mg/kg of DOX in 0.9 % normal saline intraperitoneally for 14 days after receiving oral pretreatments of 70 mg/kg/day, 140 mg/kg/day, and 210 mg/kg/day of *S. anomalum* diluted in distilled water, respectively. The positive control (group 6), were equally pretreated with 20 mg/kg/day of vitamin C, two hours prior to receiving intraperitoneal administration of 2.5 mg/kg of doxorubicin in 0.9 % normal saline for 14 days.

2.6. Blood sample collection

On the last day of the study (day 14), the rats were weighed and fasted overnight but allowed access to drinking water *ad libitum*. They were anesthetized with halothane vapour (1–2 %) for 20–30 seconds, their blood samples collected by cardiac puncture into plain centrifuge tubes, immediately centrifuged (2500 rpm for 15 minutes), and their sera used for biochemical assays. The rats' testes were then identified, harvested, and weighed and the dead animals were judiciously buried.

Table 1
The effect of *S. anomalum* leaf extract on testes' oxidative stress markers of rats with DOX-induced toxicity.

Parameters/ Treatment	Dose mg/ kg	SOD (U/ml)	CAT (U/ g of protein)	GPx (μg/ml)	GSH (μg/ ml)	MDA (μmol/ ml)
Normal control	-	0.55± 0.02	1.52 ±0.06	0.074 ±0.02	1.60 ±0.05	0.21 ±0.012
Negative control (DOX)	2.5	0.20 ±0.01[d]	0.52± 0.01[d]	0.032 ±0.001[d]	0.51 ±0.01[d]	0.68 ±0.01[d]
Positive control (Vitamin C+DOX)	20	0.44 ±0.01[c]	1.52 ±0.11[c]	0.068 ±0.001[c]	1.51 ±0.01[c]	0.38 ±0.01[c]
Extract+DOX	70	0.40 ±0.01[b]	0.62 ±0.00	0.039 ±0.00[c]	0.85 ±0.02[c]	0.40 ±0.03[c]
Extract+DOX	140	0.41 ±0.04[b]	0.65 ±0.01[b]	0.040 ±0.00[c]	0.88 ±0.01[c]	0.32 ±0.01[c]
Extract+DOX	210	0.50± 0.04[c]	1.47 ±0.12[c]	0.057 ±0.00[c]	1.27 ±0.01[c]	0.31 ±0.04[c]

Data are expressed as mean ± SEM. Significant at [d]p<0.001 when compared with normal control; [a]p< 0.05, [b]p< 0.01, [c]p< 0.001 when compared with the negative control, n = 6.

2.7. Evaluation of progressive motility, viability, count, and the structural abnormality of sperm

In order to collect the sperm samples, the caudal portion of the epididymis was isolated. The epididymal portion was first chopped finely and placed in 5 ml of physiological saline. This mixture was then incubated for 30 minutes at 37 °C to induce spermatozoa release from the epididymal ducts. Through a 400X phase-contrast microscope, sperm increasing motility percentage was found [15]. By using Eosin or Nigrosin staining, along with microscopic analysis, the viability of the sperm was determined. Additionally, epididymal sperm in the suspension were counted using a hemocytometer [16]. The Filler [17] approach was used to calculate the percentage of sperm with head, tail, and mid-piece morphological defects. The size and shape of spermatozoa heads (large or tiny heads with lighter and emphasized curvature), abnormalities in intermediary components that cause untied heads, and defects in tails (short, numerous, folded, and broken tails) were among the traits that appeared to be abnormal.

2.8. Biochemical assays

2.8.1. Lipid profile

The collected blood samples (2 ml) from the heart chamber were allowed to clot before being separated into sera and clotted blood samples using a centrifuge at 5000 rpm for 5 minutes. The biochemical parameters assayed with the aid of colorimetric techniques were serum testosterone, cholesterol, triglycerides (TG), and high-density lipoprotein (HDL-C) [18]. Moreover, from the guidelines of the respective manufacturer, the lipid parameters' assessments were carried out spectrophotometrically with Fortress Diagnostic Kits®, UK, whereas the matching rat enzyme-linked immunosorbent assay kits (Testosterone: ab108666, Abcam, Cambridge, UK) was used to determine the levels of serum testosterone.

2.9. Effect of the ethanol leaf extract on testis oxidative stress markers

The oxidative markers' assay was performed on testes homogenates of rats used in this study. These oxidative stress markers were used to

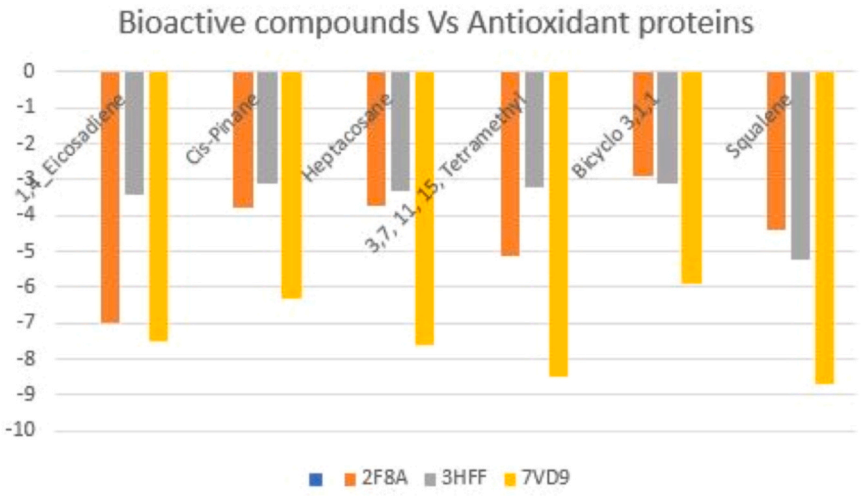

Fig. 1. A graphical plot showing the effectiveness of bioactive compounds against selected proteins.

Table 2
The effect of leaf extract of *S. anomalum* on seminal analyses of rats with DOX-induced testicular toxicity.

S/No.	Normal control	Negative control (DOX)	Positive control (Vitamin C)	Extract 70 mg/kg	Extract 140 mg/kg	Extract 210 mg/kg
Appearance	Milky	Milky	Milky	Milky	Milky	Milky
Volume	0.03 ml	0.01 ml	0.02 ml	0.02 ml	0.03 ml	0.04 ml
pH	8.0	8.0	8.0	8.0	8.0	8.0
Viability	80 %	55 %	90 %	75 %	80 %	90 %
Viscosity	Normal	Normal	Normal	Normal	Normal	Normal
Normal	80 %	55 %	90 %	70 %	80 %	80 %
Abnormal	20 %	45 %	10 %	40 %	25 %	20 %
Active	80 %	50 %	90 %	65 %	75 %	80 %
Sluggish	10 %	10 %	5 %	10 %	10 %	10 %
Dead	10 %	40 %	5 %	35 %	15 %	10 %
Sperm	500	200	600	300	450	600

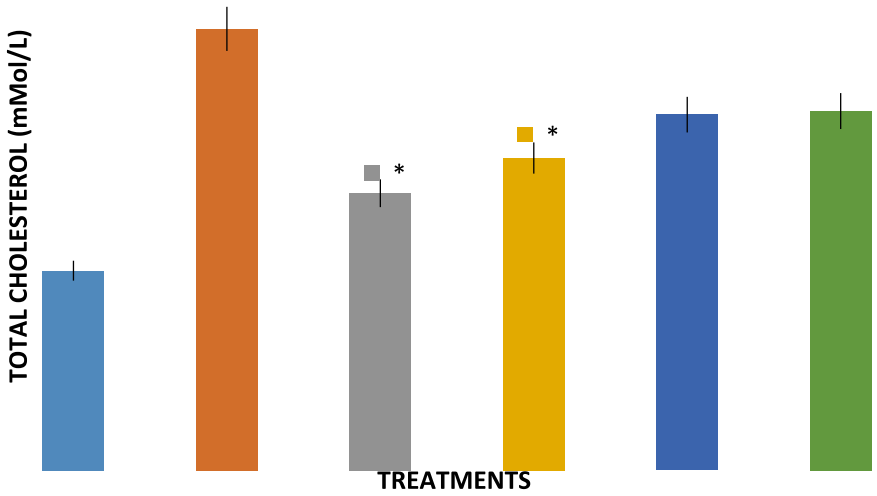

Fig. 2. Effect of the leaf extract of *S. anomalum* on total cholesterol (TC) of rats with DOX-induced testicular toxicity. Bars from left to right symbolize the following treatment groups: normal control, negative control (DOX), positive control (vitamin C+DOX), extract+DOX (70 mg/kg), extract+DOX (140 mg/kg), and extract+DOX (210 mg/kg). Data are expressed as mean ± SEM, Significant at *p<0.05, **p< 0.01, ***p< 0.001 when compared with the negative control, n=6.

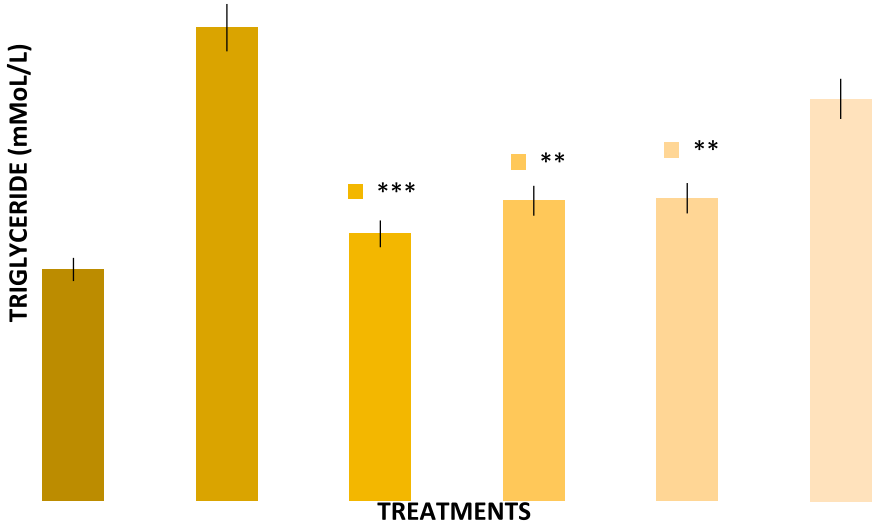

Fig. 3. Effect of the leaf extract of *S. anomalum* on TG level of rats with DOX-induced testicular toxicity. Bars from left to right symbolize the following treatment groups: normal control, negative control (DOX), positive control (vitamin C+DOX), extract+DOX (70 mg/kg), extract+DOX (140 mg/kg), and extract+DOX (210 mg/kg). Data are expressed as mean ± SEM, Significant at *p<0.05, **p< 0.01, ***p< 0.001 when compared with the negative control, n=6.

assess anti-oxidative stress potentials of the ethanol extract.

2.10. Preparation of testis homogenate

The rats' testicles were surgically removed and weighed after exposure to diethyl ether (5 ml). After a quick rinse in 1.15 % KCl buffer solution, they were placed in a fresh sample bottle and kept chilled in 0.9 % NaCl. By using a motor-driven Teflon pestle, homogenates were created in a ratio of 1 g of wet tissue to 9 ml of 1.25 % KCl. The homogenates were centrifuged at 7000 rpm for 10 minutes at 4 °C, and the supernatants were used for the SOD, CAT, GPx, reduced gluthathione (GSH), and MDA assays [19], as well as other enzymes. These oxidative stress indicators were utilized to evaluate the extract's antioxidative stress capability.

2.11. Histological studies (tissue harvesting, processing, staining, and examination)

According to established protocols by Asanga et al. [21], 10 % buffered formalin-fixed excised testes were used; the fixed tissues were dehydrated through changes of graded alcohols. This was to remove excess water inherent in tissues as follows: two changes of 70 % and 95 % alcohol for a period of two hours each and two changes of 100 % alcohol (also known as absolute alcohol) for a period of two hours. The dehydrated tissues were cleaned using xylene and impregnated with two changes of paraffin wax in the oven at the temperature of 60 °C for 1 hour 30 minutes each to enable embedding. Tissues were transferred from the final wax bath to molds filled with molten wax, allowed to solidify and thereafter, properly oriented for sectioning. The paraffin block was sectioned at 5 μm after cooling the surface of the tissues using an ice bar. Ribbons were gently picked with Carmel-brush and dropped in a water bath containing water at 60 °C to enable ribbons to float, expand, and flatten out. Slides were rubbed with thymol containing egg albumin, and gently dipped into the bath to pick up the flattened-out tissue ribbons, and the slides were placed on hot plate at 68 °C [21].

Tissue sections were soaked in water, de-paraffinized in two changes of xylene, and hydrated through graded series of alcohols in descending order. After rinsing in water and staining in Haematoxylin for

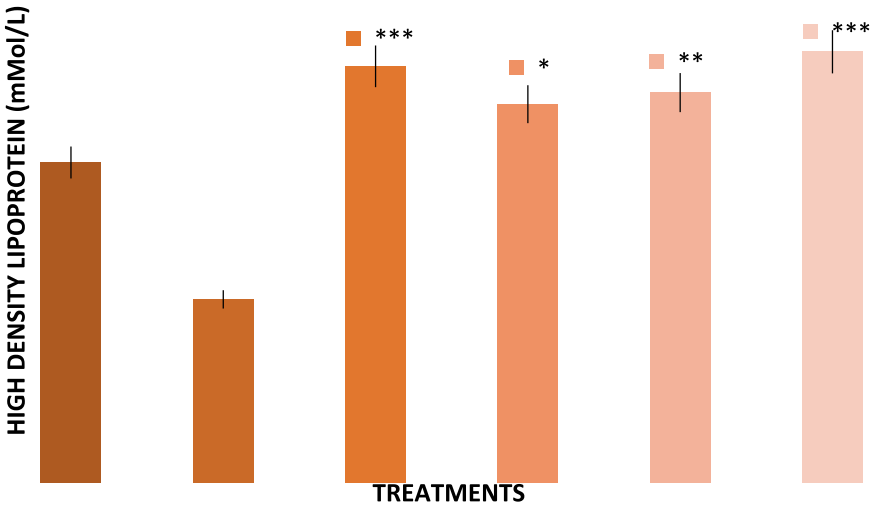

Fig. 4. Effect of the leaf extract of *S. anomalum* on HDL-C level of rats with DOX-induced testicular toxicity. Bars from left to right symbolize the following treatment groups: normal control, negative control (DOX), positive control (vitamin C+DOX), extract+DOX (70 mg/kg), extract+DOX (140 mg/kg), and extract+DOX (210 mg/kg). Data are expressed as mean ± SEM, Significant at *p<0.05, **p< 0.01, ***p< 0.001 when compared with the negative control, n=6.

Table 3
The effect of leaf extract on serum testosterone and testicular weight of rats with DOX-induced testicular toxicity.

Treatment	Dose mg/kg	Serum testosterone (ng/ml)	Testicular weight (mg)
Normal control		4.58± 0.12	2.61±0.20
Negative control (DOX)	2.5	0.84±0.06 [f]	1.33±0.05
Positive control (Vitamin C+DOX)	20	4.22± 0.17[c]	2.42±0.15
Extract+DOX	70	1.94± 0.08[b,e]	2.36±0.33
Extract+DOX	140	3.58± 0.27[c]	2.44±0.08
Extract+DOX	210	4.05± 0.33[c]	2.40±0.11

Data are expressed as mean ± SEM, Significant at [e]p<0.01; [f]p<0.001 when compared with the normal control; [a]p<0.05, [b]p< 0.01, [c]p< 0.001 when compared with the negative control, n=6.

10 minutes, sections were rinsed and differentiated in 1 % acid alcohol and blue in running water using Scot's tap water solution until sections appear blue. The blue section was counterstained in Eosin solution for 3 minutes. Tissues were washed well in water, dehydrated in ascending grades of alcohol, cleared in xylene, and mounted in DPX covered with cover slips. The sections were observed and photomicrographs taken using Leica DM 750 microscope fixed with Leica ICC50 W high-definition wireless camera module [21].

2.12. The GC-MS analysis of S. anomalum leaf

According to the procedure described by Asanga et al. [20], the ethanol extract of *S. anomalum* leaf was weighed (10 mg), diluted in dimethylsulfoxide (DMSO), and subjected to GC-MS analysis. An Agilent system made up of a model 7890 N gas chromatograph and a model Triple Quad 7000 A in EI model at 70 eV (m/z range 40–600 amu; Agilent Technologies, Santa Clara, California, USA) was injected with 2 L (split ratio 10:1; split flow 12 ml/min). A HP-5ms fused silica capillary GC column (30 m x 250 m x 0.25 m) with a 5 % phenyl-methyl polysiloxane stationary phase was employed. Helium served as the carrier gas, with a flow rate of 1.2 ml/min and a column head pressure of 9.7853 psi. The mass selective detector's inlet temperature was 250 °C. The temperature programing for the GC oven used was as follows: A starting temperature of 50 °C was maintained for 10 minutes before being raised by 6 °C per minute to 190 °C for 20 minutes and 7 °C per minute to 290 °C for 30 minutes. Based on retention indices, and by comparing mass spectrum fragmentation patterns with the National Institute of Standards and Technology (NIST) database/ChemStation data system, the chemical compounds were identified.

2.13. The assessment of the physicochemical and pharmacokinetics properties of the bioactive compounds from the ethanol extract of S. anomalum leaf

The canonical strings or Simplified Molecular-Input Line-Entry System (SMILES) strings of the various compounds were retrieved from the PubChem web platform (https://www.ncbi.nlm.nih.gov/pccompound) in 3D-conformation and were subsequently converted into mole files using MarvinSketch software (version 15.11.30). The drug-likeness and absorption, distribution, metabolism, and excretion (ADME) properties of the bioactive compounds were determined using previously reported method [36]. Potential drug-like lead compounds against the target protein were found using the SWISSADME program.

2.14. Target protein properties and molecular docking of the bioactive compounds

The Research Collaboratory for Structural Bioinformatics (RCSC) protein database was used to acquire the 3-dimensional structures of the target proteins. The following details of the recovered proteins were noted: Source: RCSC-PDB, Protein Name: glutathione peroxidase, PDB ID: 2F8A; source: RCSC-PDB; protein name: superoxide dismutase; PDB ID: 3HFF; and source: RCSC-PDB; name of the protein: catalase; PDB ID: 7VD9. The ligands were docked blindly against their target proteins. First, the water molecules in the proteins were removed. The proteins were further prepared by adding hydrogen atoms using 3D protonation, and any incomplete charges were corrected and the energy of the proteins were minimized. Next, the Site Finder MOE module located the allosteric site previously determined using the Allosite Pro online server. Biovia discovery studio software was used for visualization of the interaction. The molecular docking between the ligands and the proteins were performed using the AutoDock Vina 4.2 tool. For comparison, vitamin C was used as antioxidant drug, all of the 3-D binding contacts' (binding interactions), amino acid residues, and binding poses were recovered [36].

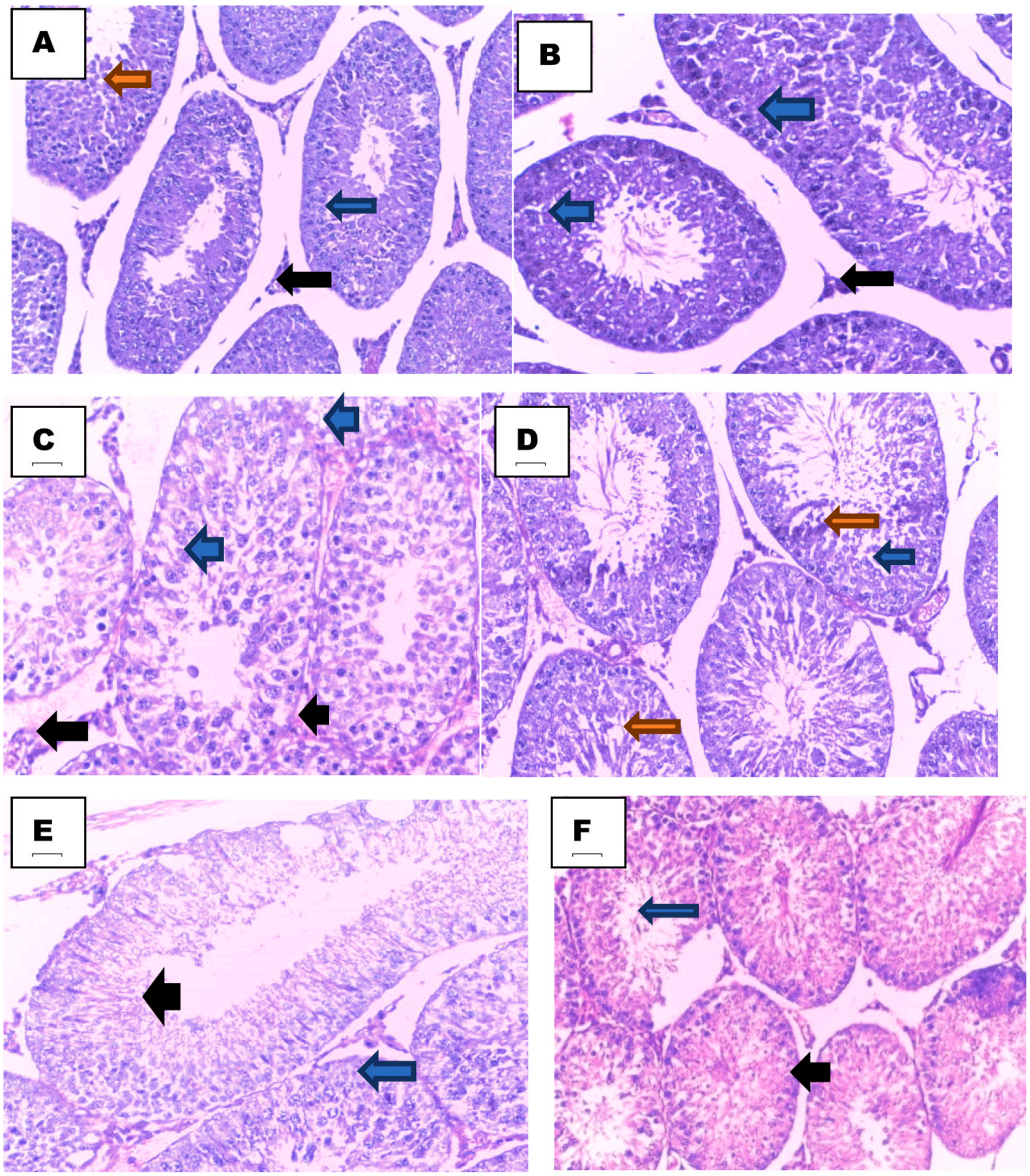

Fig. 5. Histological sections of testes of rats (A) treated with distilled water (normal control) showing normal seminiferous tubules containing numerous germ cells (blue) with normal spermatozoa (red) at the center of the tubules. Normal leydig cells seen (black); (B) Negative control group treated with DOX (2.5 mg/kg) alone showing seminiferous tubules containing degenerating germ cells and spermatozoa (blue). Normal leydig cells seen (black); (C) treated with 70 mg/kg of *S. anomalum* extract and DOX showing seminiferous tubules containing degenerating germ cells and spermatozoa (blue). Mildly hyperplastic leydig cells are seen (black); (D) treated with 140 mg/kg of *S. anomalum* extract and DOX showing normal seminiferous tubules containing numerous germ cells (blue) with normal spermatozoa (red) at the center of the tubules; and (E) treated with 210 mg/kg of *S. anomalum* extract and DOX showing normal seminiferous tubules containing numerous germ cells (blue) with normal spermatozoa (red) at the center of the tubules; and (F) Positive control group treated with Vitamin C (40 mg/kg) and DOX showing normal seminiferous tubules containing numerous germ cells (blue) with normal spermatozoa (red) at the center of the tubules.

2.15. Statistical analyses

One-way analysis of variance (ANOVA) and Tukey's multiple comparison post-test were used to examine the data (Graph Pad Prism Software Inc. La Jolla, CA, USA). Significant differences from the control group were assessed at p0.001 and p0.05. Values were given as mean ± SEM (standard error of mean).

3. Results

3.1. Effect of ethanol extract of S. anomalum leaf on testes' oxidative stress markers

The administration of DOX (2.5 mg/kg i.p) for 14 days significantly ($p<0.05–0.001$) caused decreases of testes antioxidant enzymes (SOD, GPx, and CAT) and GSH levels whereas the level of MDA was elevated. However, concomitant administration of ethanol extract of *S. anomalum* leaf (70–210 mg/kg) with doxorubicin for 14 days caused marked significant ($p<005–0.01$) elevation of the enzymatic endogenous antioxidants in the treated rats when compared with the negative control group. The treatment also caused significant ($p<0.001$) reduction in the level of MDA of the treated rats when compared to negative control group (Table 1 and Fig. 1).

3.2. Effect of leaf extract of S. anomalum on seminal analysis

The result of the seminal analysis of semen (Table 2) from rats with DOX-induced testicular toxicity revealed that the semen in all the

Table 4
GC-MS analysis of the extract of *S. anomalum* leaf.

S/ No.	Compounds	Formula	Retention time (minutes)	Molecular mass (g/mol)
1	Bicyclo[3.1.1] heptanes−2,5,6-trimethylsilyl	$C_{19}H_{38}O_5Si_4$	10.044	458.18
2	Squalene	$C_{30}H_{50}$	18.315	410.39
3	Beta-sitosterol trimethylsilyl ether	$C_{32}H_{58}Si$	18.326	486.43
4	Cis-pinane	$C_{10}H_{18}$	10.044	138.25
5	3,7,11,15-tetramethyl−2-hexadecen−1-ol	$C_{20}H_{40}O$	10.524	296.1
6	1,4-Eicosadiene	$C_{20}H_{38}$	10.547	278.5
7	Heptacosane	$C_{27}H_{56}$	22.836	380.44

treatment groups was found to be milky in appearance, while the semen volume in the negative control group administered with DOX alone was found to be the least (0.01 ml). There was a marked dose-dependent improvement in the semen volume (0.02–0.04 ml) of rats treated with the extract and the mean percentage of hydrogen (pH) of the semen samples from all the groups was 8.0. The extract-treated groups were found to have higher percentages of viable sperm cells (75–90 %) compared to the negative control group (55 %). Vitamin C (positive control) treated group had 90 % viable cells while that of the normal control was 80 %. Viscosity of semen in all the groups was normal whereas the percentage of active sperm cells in the extract-treated groups dose-dependently ranged from 65 % to 80 % as compared to 55 % recorded in the negative control group. The percentage of dead sperm cells in the negative control group was found to be about 40 % compared to 35 % in the low dose (70 mg/kg) group, 15 % in middle dose (140 mg/kg) dose treated group and 10 % in the high dose (210 mg/kg) treated-group. The positive control (Vitamin C) group had 10 % dead sperm cells. The sperm count for the negative control group was 200 cells, while 300, 450 and 600 cells were recorded from 70, 140, and 210 mg/kg treated groups, respectively. The positive control as well as the normal control groups had sperm counts of 500 and 600 cells, respectively (Table 2).

3.3. Hypolipidemic effect of leaf extract on lipid profile of rats with DOX-induced toxicity

The administration of DOX (2.5 mg/kg, i.p) for 14 days to rats caused significant increases in the levels of lipid profile parameters (total cholesterol (TC) and TG) of rats ($p<0.05$), while HDL-C level was reduced as observed in the negative control group. Concomitant treatment of rats with the extract of *S. anomalum* (70–210 mg/kg) and DOX caused significant ($p<0.05$) decreases in the levels of elevated TC and TG when compared with the negative control group, and HDL-C level was significantly ($p<0.05$) increased in the extract treated groups (Figs. 2- 4).

3.4. Effect of the leaf extract on serum testosterone level of rats with DOX-induced testicular toxicity

Administration of DOX (2.5 mg/kg i.p) for 14 days was found to significantly ($p<0.001$) cause decreases of the serum testosterone levels of rats when compared to normal control. However, the concomitant administration of the ethanol extract of *S. anomalum* (70–210 mg/kg) and vitamin C with DOX for 14 days caused significant ($p<005$–0.01) elevation of the testosterone levels of the treated rats when compared with the negative control groups (Table 3).

3.5. Effect of the leaf extract of S. anomalum on histology of testes from rats with DOX-induced testicular toxicity

The treatment of rats with the extract of *S. anomalum* (70–140 mg/kg) was found to protect the testes of rats from morphological damage. The testes of rats treated with DOX alone was found to have marked degenerated germ cells and spermatozoa, while those treated with the low dose (70 mg/kg) had mild degeneration of cells. The testes of rats treated with higher doses of the leaf extract (140 and 210 mg/kg) and vitamin C were found to be normal (Fig. 5).

3.6. The Gas chromatography mass spectrometry (GC-MS) analysis of the extract of S. anomalum leaf

The GC-MS result (Table 4) revealed the names, retention time,

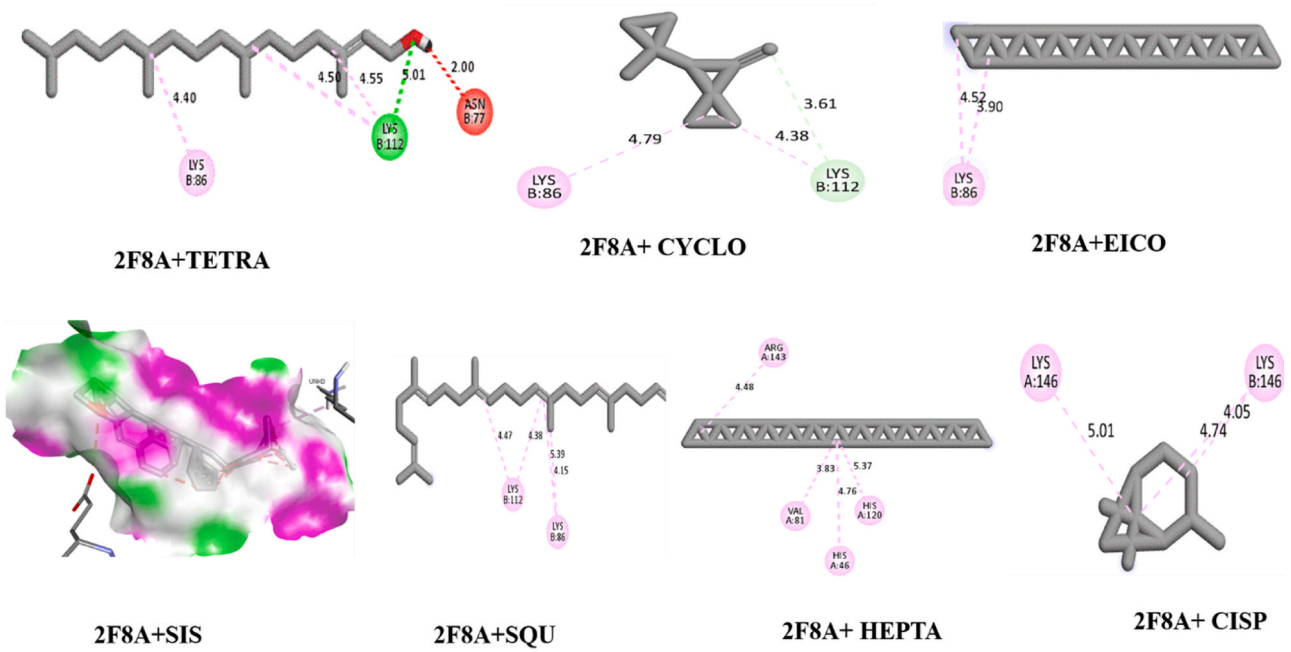

Fig. 6. Docking of ligands with glutathione peroxidase (2F8A).

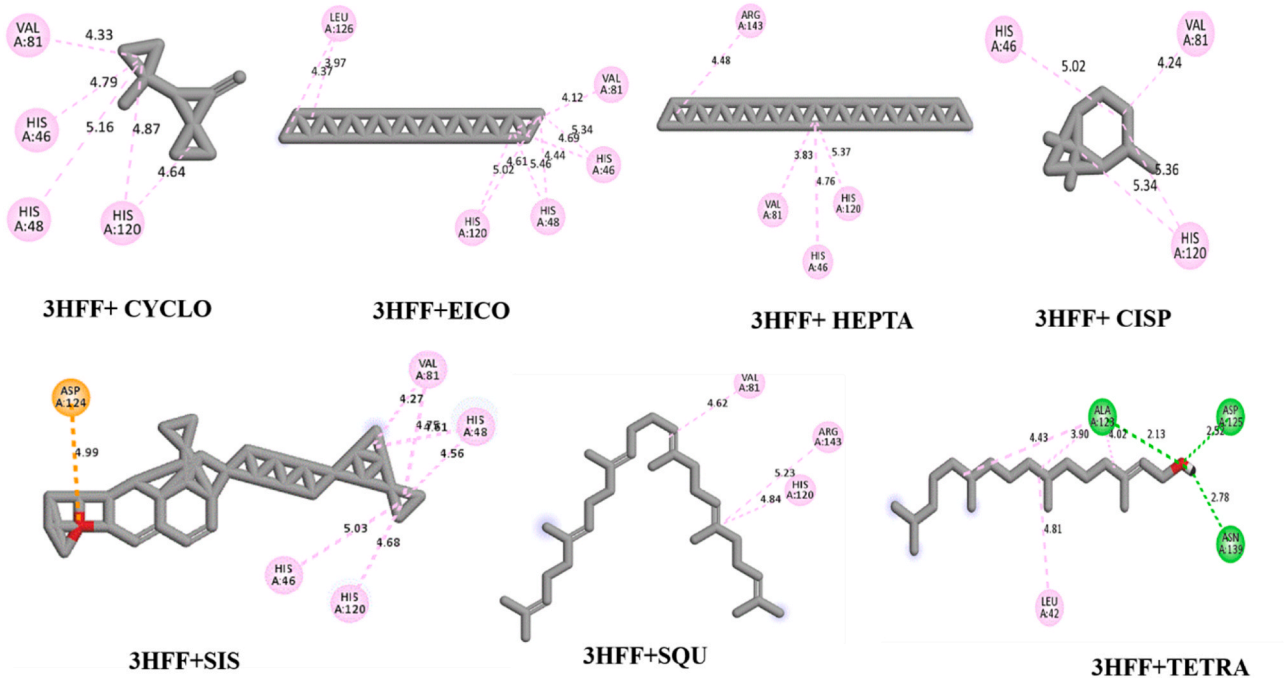

Fig. 7. Docking of ligands with superoxide dismutase (3HFF).

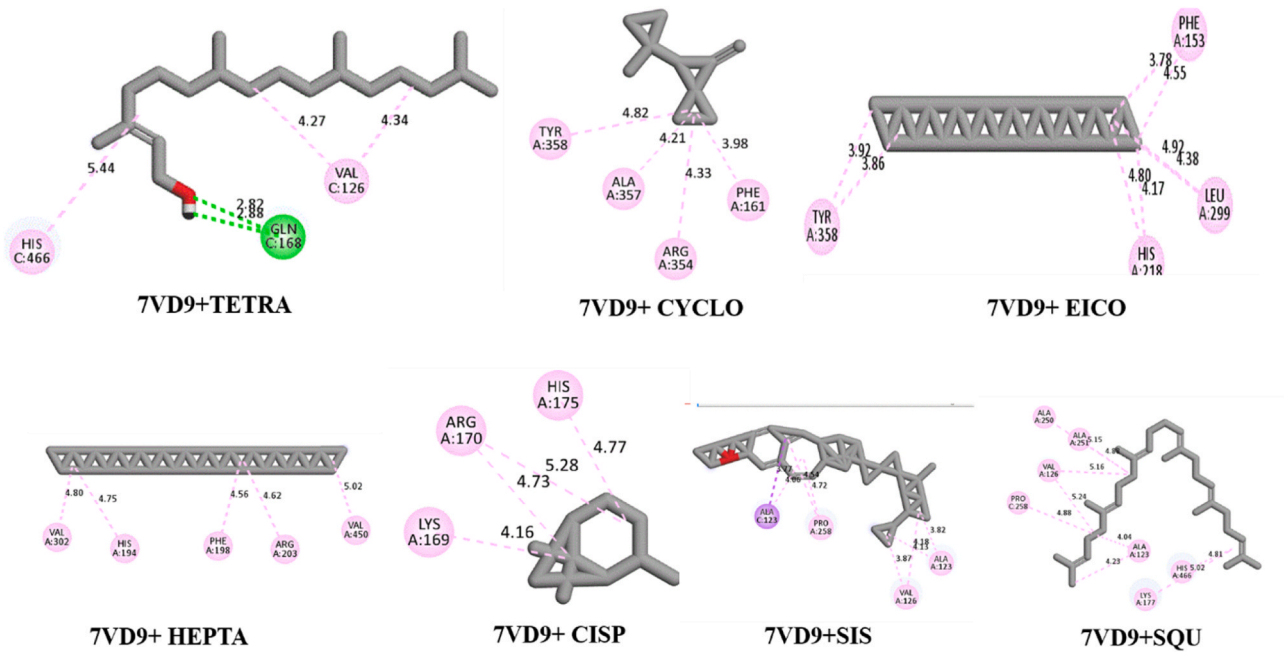

Fig. 8. Docking of ligands with catalase (7VD9), Key: TETRA = 3,7,11,15-tetramethyl-2-hexadecen-1-ol; EICO = 1,4-Eicosadiene; CYCLO = Bicyclo[3.1.1]heptanes-2,5,6-trimethylsilyl; CISP = Cis-pinane; HEPTA = Heptacosane; SIS = Beta-sisterol trimethyl ether; SQU = Squalene.

formula, and the molecular mass of the following compounds: 1,4-Eicosadiene; bicyclo[3.1.1]heptanes-2,5,6-trimethyl-(1.alpha 2-beta, 5alpha); squalene; beta-sitosterol trimethylsilyl ether; 3,7,11,15-tetramethyl-2-hexadecen-1- ol, cis-pinane; and heptacosane as the bioactive compounds characterized from the *S. anomalum* leaf.

3.7. Docking of the ligands with the target proteins

The GPx, SOD, and CAT proteins docked successfully with the various ligands (Figs. 6–8). According to the results, the amino acid residues and their binding affinities were adequately extracted and reported (Table 5 and Fig. 1). Their molecular interactions with the various amino acids' residues in the protein binding pockets revealed non-covalent bonding such as hydrogen, hydrophobic, and electrostatic bonds.

3.8. Physicochemical, pharmacokinetics, and drug-likeness properties of the various ligands

The result (Table 6) revealed that all the compounds had low absorption rate from the gastrointestinal tracts (GIT), with 14.3 % showing abilities to permeate the blood-brain barrier. 57.1 % were not substrates

Table 5
Binding affinities of studied ligands against proteins: 2F8A, 3HFF, and 7VD9.

Bioactive compounds	Binding affinities		
	Glutathione peroxidase (2F8A)	Superoxide dismutase (3HFF)	Catalase (7VD9)
1,4-Eicosadiene	−7.0	−3.4	−7.6
Cis-Pinane	−3.8	−3.1	−6.3
Heptacosane	−3.7	−3.3	−7.6
3,7,11,15-tetramethyl−2-hexadecen−1-ol	−5.1	−3.2	−8.5
bicyclo[3.1.1] heptanes−2,5,6-trimethyl-(1.alpha 2-beta, 5alpha-	−2.9	−3.1	−5.9
Squalene	−4.4	−5.2	−8.7

of permeation glycoprotein, and non-inhibition of cytochrome P450 enzymes (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4) were (85.7 %), (100 %), (71.4 %), (100 %), and (100 %), respectively. The non-violation of Lipinski rules had higher bioavailability scores of 0.55 and 0.85. On physicochemical properties (Table 6), beta-sitosterol trimethylsilyl ether had the highest molecular weight of 539.30 g/mol. Log Kp, indicates the ability of a compound to cross cell membranes, Cis-Pinane demonstrated a higher Log Kp value of −4.37 cm/s whereas 1,4 Eicosadiene had comparatively low value of −1.01 cm/s as compared to the other compounds. In addition, synthetic availability indicates the ease of a ligand to undergo drug synthesis, with beta-sitosterol trimethylsilyl ether having the highest value of 7.58 whereas bicyclo [3,1,1] heptanes-2,5,6-trimethylsilyl had the lowest value of 2.43, proving that beta-sitosterol trimethylsilyl ether can easily yield more synthetic products.

4. Discussion

Doxorubicin, as an anthracycline antibiotic is already known to slow spermatogenesis and testicular growth, thereby resulting in male infertility and the induction of oxidative stress, as well as cellular apoptosis [22]. However, the effects of DOX-induced metabolites noted for their abysmal generation of ROS and attendant pathogenesis could be counteracted with antioxidants from either endogenous or exogenous sources. Therefore, this study has provided evidence that the bioactive compounds in *S. anomalum* leaf could attenuate DOX-induced toxicity in the male reproductive system of rats. It was discovered that DOX significantly increased the percentage of aberrant and dead cells, reduced seminal volume and sperm counts as well as deteriorated spermatozoa and germ cells in the testes. However, these testicular toxicity evidences were reduced after concurrent administration of the *S. anomalum* extract as animals had higher sperm cell counts, larger seminal volumes, lower level of dead cells, and normal testicular histoarchitecture, giving credence to the bioactive compounds' potential in the reduction of DOX-induced testicular toxicity. This may be due to the anti-oxidative stress activity of 1,4-Eicosadiene; bicyclo[3.1.1] heptanes-2,5,6-trimethyl-(1.alpha 2-beta, 5alpha); squalene; beta-sitosterol trimethylsilyl ether; 3,7,11,15-tetramethyl-2-hexadecen-1- ol, cis-pinane, and heptacosane compounds. It is worthy of note that squalene ($C_{30}H_{50}$) as a polyunsaturated hydrocarbon is reported to have antioxidant and anticancer activities [23,24], antimicrobial and anticancer activities [25], as well as anti-inflammatory [26]. More so, 3, 7,11,15-tetramethylhexadec-2-en-1-ol is a diterpene with long-chain fatty alcohol and its antioxidant potential may contribute to skin health and anti-aging effects, thereby helping in the protection against oxidative stress and UV-induced damage [27]. Also, β-sitosterol is one of the various phytosterols with roles as sterol methyltransferase inhibitor, an anticholesterolemic drug and an antioxidant [28]. Moreover, Che et al. [29] reported that bicyclo[3.1.1]heptanes-2,5,6-trimethyl-(1. alpha 2-beta, 5-alpha) possesses antioxidant, antimicrobial, anti-inflammatory, antitumour, and chemo-preventive activities. The antioxidative stress activity observed in this study could arise because of these antioxidant compounds; therefore, this report showed consistency with that earlier reported by Offor et al. [30] on *S. anomalum* reducing lead-induced toxicity and improving sperm quality.

Howbeit, ROS easily attacks polyunsaturated fatty acids that are abundant in the membranes of male germ cells [31], so, spermatozoa are vulnerable to oxidative damage, which causes them to lose their

Table 6
Physicochemical, pharmacokinetics, and drug-likeness properties for the bioactive compounds.

Parameters	1,4 Eicosadiene	3,7,11,15-tetramethyl-2-hexadecen-1-ol	Bicyclo [3.1.1] heptanes-2,5,6-trimethylsilyl	Cis-pinane	Heptacosane	Beta-sisterol trimethyl ether	Squalene
Physicochemical properties							
	$C_{20}H_{38}$	$C_{20}H_{40}O$	$C_{10}H_{28}$	$C_{10}H_{18}$	$C_{27}H_{56}$	$C_{32}H_{108}OSi$	$C_{30}H_{50}$
Molecular weight(g/mol)	278.52	296.56	148.33	138.25	380.73	539.30	410.72
No. heavy atoms	20	21	10	10	27	34	30
No. rotatable bonds	16	13	0	0	24	0	15
H-bond acceptor	0	1	0	0	0	1	0
H-bond donor	0	1	0	0	0	1	0
TPSA (Å²)	97.34	20.23	0.00	0.00	0.00	9.23	0.00
Pharmacokinetics							
GI absorption	Low	Low	Low	Low	Low	Low	
BBB permeant	No	No	No	Yes	No	No	
P-gp substrate	No	Yes	Yes	No	Yes	Yes	
CYP1A2 inhibitor	Yes	No	No	No	No	No	
CYP2C19 inhibitor	No	No	No	No	No	No	
CYP2C9 inhibitor	No	Yes	No	Yes	No	No	
CYP2D6 inhibitor	No	No	No	No	No	No	
CYP3A4 inhibitor	No	No	No	No	No	No	
Log Kp (skin permeation) (cm/s)	−1.01	−2.29	−3.31	−4.37	1.49	3.74	
Drug-likeness							
Lipinski	Yes; 1 violation	Yes 1 violation	Yes; 1 violation	Yes;1 violation	Yes;1 violation	No; 2 violations	
Ghose	No;1 violation	No; 1 violation	No; 1 violation	No: 1 violation	No: 3 violations	No; 4 violations	
Veber	No; 1 violation	No; 1 violation	Yes	Yes	No; 1 violation	Yes	
Bioavailability score	0.55	0.55	0.55	0.55	0.55	0.17	
Synthetic availability	3.83	4.30	2.43	4.03	3.56	7.58	

integrity and become less mobile. Therefore, the low sperm count, large percentage of dead, and aberrant cell counts observed in this study for spermatozoa and markedly degraded germ cells in the testes' histological sections could have resulted from ROS attack on the membrane of the testes because of DOX-induced testicular toxicity. However, the rats treated with the *S. anomalum* ethanol extract expressed minor toxicity, an indication of a possible testicular protective effect of the compounds (beta-sitosterol and squalene) known for their anticholesterolemic and antioxidant activities.

Moreover, a crucial component of the reproductive system is lipids, known to be very critical in both steroidogenesis and spermatogenesis. Takashima et al. [32] and Das et al. [33] had reported that DOX could down regulate testosterone levels with negative implications on the number, motility, and viability of spermatozoa. According to this study, testosterone level was considerably lower in the negative control as compared with the normal control group, however, higher in all other treatment groups, further supporting the plant extract's ability to protect against DOX-induced damages.

Nevertheless, ROS produced as a result of oxidative stress, are crucial in the development of DOX-induced toxicity [1]. Further evaluation in this study in comparison with the other treatment groups revealed that the negative control group had considerable increase in testicular MDA level and significant decreases in testicular SOD, CAT, GPx, and GSH. These findings showed consistency with the report by Khodir et al. [34] and Nowrouzi et al. [35] that DOX-induced testicular oxidative stress could lead to deficient antioxidant defense system. However, the co-treatment with the extract and DOX increased the levels of these endogenous antioxidants, revealing the leaf extract's potential in free radical scavenging; hence, its antioxidative stress activity could be related to the presence of 1,4-Eicosadiene; bicyclo[3.1.1]heptanes-2,5,6-trimethyl-(1.alpha 2-beta, 5alpha); squalene; beta-sitosterol trimethylsilyl ether; 3,7,11,15-tetramethyl-2-hexadecen-1- ol, cis-pinane, and heptacosane compounds.

Furthermore, SOD, CAT, and GPx are three antioxidant proteins that play awesome roles in the body's defense against ROS, being dangerous chemicals that could destroy cells and promote the pathogenesis of several diseases [21]. The analysis from this study revealed that the best three compounds based on their binding affinities in decreasing order of magnitude were 1,4-Eicosadiene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, and squalene, respectively. Similarly, the docking of ligands with SOD and CAT revealed squalene, 1,4-Eicosadiene, and 3,7,11,15-tetramethyl-2-hexadecen-1-ol, as well as squalene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, and 1,4-Eicosadiene, in decreasing order of magnitudes, respectively. Therefore, squalene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, and 1,4-Eicosadiene facilitated electrostatic and hydrophobic interactions with the various amino acid residues in the target proteins' binding pockets; hence, the reason for their low binding energies and docking scores. This observation was similar to the report by Asanga et al. [36] that lead compounds had lower docking energy scores; hence, lower score function during docking simulations is an advantage for the prediction of both strength and type of signals produced during the structure-based design of drugs.

In addition, the evaluation of the pharmacokinetics of the ligands showed that all the compounds had lower rates of absorption in the gastrointestinal tract (GIT), and were non-substrates for permeation glycoprotein and non-inhibitors of monooxygenases. More so, these compounds except beta-sitosterol trimethylsilyl ether obeyed Lipinski rules of five; therefore, the lead compounds portraying these acceptable physicochemical and pharmacological properties imply they are potential drugs.

5. Conclusion

The study demonstrated that the extract of *S. anomalum* leaf contains squalene, β-sitosterol, cis-pinane, 1,4-Eicosadiene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, heptacosane, and bicyclo-heptanes-2,5,6-trimethylsilyl. These compounds potentially attenuated the testicular toxicity induced by doxorubicin because they possess remarkable antioxidative, antioxidant, hypolipidemic, and improved testicular histoarchitecture. Therefore, these compounds have antidotal potentials. Moreover, these compounds revealed remarkable binding energies, pharmacokinetics, physicochemical, and drug-likeness potentials; hence, could be used for the treatment of doxorubicin-induced testicular pathologies.

Ethical approval

The NIH protocols for handling, use, and care of experimental animals (NIH publication No. 8023, amended 1978) were well followed. The department's animal ethics committee gave its approval (approval number: MUEPHARM/0126) and made sure the experimental protocol was strictly followed.

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CRediT authorship contribution statement

Jude Okokon: Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology. **Akaninyene Joseph:** Writing – review & editing, Writing – original draft, Validation, Software, Conceptualization. **Chinedum Ekeleme:** Writing – review & editing, Supervision, Resources, Investigation, Formal analysis, Data curation. **Edet Asanga:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Somto Ilechukwu:** Writing – review & editing, Validation, Project administration, Methodology. **Martin Anagboso:** Writing – review & editing, Resources, Project administration, Formal analysis. **Mercy Umoh:** Writing – review & editing, Resources, Methodology, Formal analysis, Data curation. **Atim-Ebim Raymond:** Writing – review & editing, Visualization, Validation, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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