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Research article

An *In vitro* and *in silico* investigation of the antitrypanosomal activities of the stem bark extracts of *Anopyxis klaineana* (Pierre) Engl

Latif Adams [a,b,*], Dorcas Obiri-Yeboah [b], Michael Afiadenyo [c], Sherif Hamidu [d], Abigail Aning [d], Ebenezer Ehun [e], Katie Shiels [f], Akanksha Joshi [f], Maxwell Mamfe Sakyimah [e], Kwadwo Asamoah Kusi [g], Irene Ayi [c], Michelle Mckeon Bennett [a], Siobhan Moane [a,**]

[a] *Technological University of Shannon: Midlands Midwest, Midlands Campus, Athlone, Ireland*
[b] *Department of Microbiology and Immunology, School of Medical Sciences, College of Health and Allied Sciences, University of Cape Coast, Cape Coast, Ghana*
[c] *Department of Parasitology, Noguchi Memorial Institute for Medical Research (NMIMR), College of Health Sciences (CHS), University of Ghana, Legon, Accra, Ghana*
[d] *Department of Clinical Pathology, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, P.O. Box LG 581, Legon, Ghana*
[e] *Centre for Plant Medicine Research, Mampong-Akuapem, Ghana*
[f] *Shannon Applied Biotechnology Centre(ABC), Technological University of Shannon, Moylish Park, Limerick, Ireland*
[g] *Department of Immunology, Noguchi Memorial Institute for Medical Research (NMIMR), College of Health Sciences (CHS), University of Ghana, Legon, Accra, Ghana*

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ABSTRACT

African Trypanosomiasis caused by trypanosome parasites continues to be a major neglected health problem, particularly in developing countries. Current treatments are marked by serious side effects, low effectiveness, high toxicity, and drug resistance prompting the need to develop novel, safe, effective, and alternative antitrypanosomal compounds. *Anopyxis klaineana* is an ethnomedicinal plant used in West Africa to treat many ailments including protozoan diseases. In this study, we investigated the antitrypanosomal potential of stem bark extracts of *A. klaineana* through *in vitro* and *in silico* approaches. *A. klaineana* extracts were tested for their anti-trypanosomal activities against *Trypanosoma brucei* parasite *in vitro* using Alamar blue assay. In addition, the antioxidant and cytotoxic activities were determined. LC-ESI-QTOF-MS was used to identify potential bioactive compounds present in the *A. klaineana* extracts. Bioactive compounds identified were subjected to molecular docking studies against *Trypanosoma brucei's trypanothione reductase* (TR) and Uridine Diphosphate Galactose 4′-Epimerase (UDP). The *A. klaineana* extracts (methanol, hexane, chloroform, and ethyl acetate) exhibited potential anti-trypanosomal activities with IC_{50} values of 21.25 ± 0.755,4.35 ± 0.166,2.57 ± 0.153 and 22.92 ± 2.321 μg/mL respectively. Moreover, the methanolic crude extracts showed moderate cytotoxicity against

* Corresponding author. Technological University of Shannon: Midlands Midwest, Midlands Campus, University Road, Athlone, Co. Westmeath, Ireland.
** Corresponding author. Technological University of Shannon: Midlands Midwest, Midwest Campus, University Road, Athlone, Co. Westmeath, Ireland.
E-mail addresses: latifadams2016@yahoo.com (L. Adams), siobhan.moane@tus.ie (S. Moane).

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HepG2 and PNT2 cells, with IC_{50} values of 68.0 ± 2.05 and 78.7 ± 2.63 μg/mL respectively. LC-MS analysis revealed the presence of 24 bioactive compounds with 5 being druglike. Risperidone, Ranolazine, Dihydro-7-Desacetyldeoxygedunin, 6 beta-Hydroxytriamcinolone acetonide, and Dimethylmatairesinol were identified as novel potential inhibitors of TR and UDP with binding affinities of −10.4, −7.9, −8.7, −8.4 and −7.1 kcal/mol respectively against TR and −10.8, −8.4, −8.4, −7.6 and −8.1 respectively against UDP. This study indicates that *A. klaineana* has potential antitrypanosomal properties and therefore may have the potential to be developed as a therapeutic intervention for treating African trypanosomiasis.

1. Introduction

Protozoan diseases continue to be a major public health concern worldwide, especially in developing countries and they are associated with high significant morbidity and large economic impacts [1–3]. Among these, African trypanosomiasis, widely known as sleeping sickness, is a Neglected Tropical Disease (NTD) transmitted by the tsetse fly and caused by *Trypanosoma species*. It is endemic in sub-Saharan African countries and mostly affects both humans and animals in poor rural areas. African trypanosomiasis includes Human African trypanosomiasis (HAT) and African Animal trypanosomiasis (AAT) [4]. HAT involves two forms caused by two different subspecies of the parasite which include *T. brucei gambiense* present in Western and Central Africa, while *T. brucei rhodesiense* is present in Eastern and Southern Africa [5].

Currently, about 60 million people are at risk of this infection [6] whereas AAT hinders the production of livestock especially cattle which is a major setback to the economic development of most affected areas [7]. In the absence of vaccine due to antigenic variation displayed by the trypanosome parasites, chemotherapy remains the most viable and efficient option for treatment or control of African trypanosomiasis [8]. HAT is treated with Pentamidine, Suramin, Melarsoprol, Eflornithine, Nifurtimox and Fexinidazole depending on the stage of the disease [9]. AAT is usually treated with Diminazene aceturate, Isometamidium and Homidium [10]. However, these drugs currently in use have serious side effects, highly toxic and less effective due to resistance developed by parasite [11–15]. Hence, there is urgent need for the development of novel, alternative, effective and safe compounds to treat this disease.

Medicinal plants have consistently gained much attention as potential and an alternative source for development of African trypanosomiasis chemotherapy [16,17]. Many studies have reported the use of medicinal plants to treat African trypanosomiasis [18,19]. *Anopyxis klaineana* (Pierre) Engl is a semi-deciduous tree that belongs to the family Rhizophoraceae and widely distributed in West Africa. It is locally known as *kokoti* and *bodioa* in Ghana and Ivory Coast respectively. *A. klaineana* is ethnomedicinally used to treat several ailments and diseases. Different parts of this plant are employed in traditional medicine. The stem bark decoction is used to treat sexually transmitted diseases, joint pains, kidney disorders, pneumonia, bronchitis and stomach disorders In addition, the powdered bark is applied topically for treating, wounds and skin infection [20].

In Southern Ghana, local communities around Kakum National park uses the stem bark decoction of *A. klaineana* to treat malaria [21]. In-addition, recent studies by Essandoh PK et al. also reported that local villages around Ankasa Forest Reserve, Western Ghana uses both the bark and root to treat malaria [22]. The stem bark decoction of this plant is also reported to be used in treating schistosomiasis in Atwima Nwabiagya district in Ghana [23]. Studies on biological activities of this plant have demonstrated anti-inflammatory [24,25], Neuropharmacological [26] and Cercaricidal activities [27].

Owing to the ethnopharmacological usage of this plant to treat several parasitic diseases, we sought explore the antitrypanosomal potential of the stem bark of *A. klaineana* and its possible development as a therapeutic intervention for treating HAT through *in-vitro* investigations. Moreover, we also determine the mechanism of the antitrypanosomal activity of the methanolic crude extract of *A. klaineana* through *in-silico* approach. LC-MS analysis was performed to determine the potential bioactive compounds present in the methanolic crude extract of stem bark of *A. klaineana*. The compounds were then docked against *Trypanothione reductase* (TR) and Uridine Diphosphate Galactose 4′-Epimerase (UDP) since they play a major role in the survival of the parasite hence inhibiting them will result in the death of the parasites [28,29]. Molecular docking provides first line of proof for inhibition by determining the binding affintiy between molecules and the binding pose of a ligand in the binding pocket of a macromolecule [30,31]. *T. brucei* UDP is a dimeric short-chain dehydrogenase/reductase that synthesizes galactose from glucose using hexose transporters [32]. In order to produce UDP-galactose, UDP inverts the chirality of the C4 carbon atom in UDP-glucose. The two-step process advances through a transient ketose intermediate and necessitates rotation of the ligand's sugar component in order to epimerize [32,33]. *T. brucei's* bloodstream form has a lot of glycoproteins that contain galactose, but unlike mammalian cells, the parasite's hexose transporters are unable to readilly absorb galactose. *T. brucei* must instead rely on the enzyme's action to receive its galactose. UDP has been shown to be necessary for the survival of the parasite in both the disease-relevant bloodstream form and the Tsetse-fly dwelling procyclic form by the generative activity of a *T. brucei* conditional null genetic mutant [32,33].

Trypanothione reductase (TR) is a crucial enzyme in the defense mechanisms of infectious trypanosomatids, which rely on dihydro-trypanothione to maintain thiol homeostasis as they lack essential redox catalyse system [34,35]. TR, an NADPH-dependent disulfide oxidoreductase, converts trypanothione disulfide into dihydro-trypanothione, which protects trypanosomatids from oxidative damage [28,35] Studies have shown that inhibitors interupt the activity of TR, making it an ideal target for developing drugs to combat trypanosomatidae diseases, offering a promising avenue for therapeutic intervention [28].

2. Material & methods

2.1. Chemicals and reagents

Methanol, Hexane, Ethyl Acetate, Chloroform, Ferric Reducing Antioxidant Power (FRAP) assay kit, Dulbecco's Modified Eagle Medium (DMEM), Dulbecco's phosphate-buffered saline (DPBS), were purchased from Sigma-Aldrich. MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide), Trypsin-EDTA, Penicillin-Streptomycin, Hirumi's Modified Iscove's Medium-9 (HMI-9), Fetal Bovine Serum (FBS) were obtained from Thermo Scientific, Dublin, Ireland.

2.2. Collection and preparation of plant material

For the collection of plant material, permission was sought from the Plant Protection and Regulatory Services Directorate, Ministry of Food and Agriculture, Cape Coast, Ghana with certificate no:0040752. The stem barks of *A. klaineana* were collected in May 2022 from Abrafo (5°20′N 1°23′W), Central Region, Ghana. The plant was authenticated and identified by Dr. Isaac Henneh Tabiri, a Pharmacognosist at the University of Cape Coast and deposited with voucher number (CCG20220) at the Herbarium unit of the School of Biological Sciences, University of Cape Coast. The stem barks were washed under a running tap to remove debris and unwanted materials, cut into small pieces, air dried under ambient room temperature (20 °C) for a week, and crushed into powder with aid of an electric mill. The dried powdered samples were stored in plastic bags in a cool dry place until required for use. All the experiments were done according to the required guidelines and regulations.

2.3. Extraction of plant material

The dried powdered stem bark of *A. klaineana* was extracted using the Soxhlet technique as previously described [36]. One hundred grams (100 g) of powdered material was weighed into an extraction thimble and placed in a Soxhlet apparatus. They were extracted with 1L of 70% methanol. The resulting extract was evaporated on Eyela rotary evaporator (SB-1200). The methanolic crude extract was weighed to obtain 7.4 g and stored in vials in a refrigerator at 4 °C until used.

2.4. Partitioning of the methanolic crude extract

The crude methanolic extract of *A. klaineana* underwent sequential liquid to liquid extraction. 250 mL of the methanolic crude extract was partitioned three times with 250 mL of Hexane, Chloroform and Ethyl acetate solvents respectively. The extracts were evaporated to dryness using rotary evaporator to obtain the Hexane extract (0.2148 g), Chloroform extract (0.712 g), and Ethyl acetate extract (6.3747 g).

2.5. Preliminary phytochemical analysis

The stem bark methanolic crude extract of *A. klaineana* was preliminary screened for the presence of phytochemicals such as alkaloids, phenols, flavonoids, terpenoids, steroids, tannins, and saponins using standard procedures as previously described [37] (supplementary data).

2.6. Fourier transformed infrared spectroscopy (FITR)

FITR analysis was carried on the stem bark methanolic crude extract of *A. klaineana* to determine various functional groups present in the sample. This experiment was done by Thermo Scientific™- Nicolet™ iN10 Infrared Microscope instrument.

2.7. Liquid chromatography-mass spectrometry (LC-MS) analysis

The LC-MS analysis was determined to identify potential bioactive compounds present in stem bark methanolic extract of *A. klaineana* using an Agilent 6520 quadrupole time-of-flight mass spectrometer linked with Agilent 1200 HPLC system via dual ESI interface (Agilent Technologies, USA). A previously developed method was used for the characterization of potential bioactive compounds as reported in our recent studies [38]. Mass Hunter software version 10 (Agilent Technology) was used for data analysis.

2.8. Determination of total flavonoid content

The $AlCl_3$ colorimetric technique was utilized to evaluate the total flavonoid content present in the stem bark methanol extract of *A. klaineana* [39,40]. First, 20 mg of the extract were diluted in 1 mL of a distilled water. Two further concentrations (5 and 10 mg/mL) were achieved by using a 2-fold serial dilution. In order to generate a standard quercetin curve, 1 mg of quercetin was dissolved in 1 mL of methanol. Seven different concentrations, ranging from 0.0015625 to 0.1 mg/mL, were then prepared using a two-fold serial dilution method. One hundred microliters of each prepared extract or quercetin sample was put to a pre-labelled 96-well plate. This was followed by an equal volume of 2% $AlCl_3$. After fully combining the plate, it was left to stand at room temperature for 20 min. Using a Synergy HT Biotek microplate reader, absorbance values were measured at 415 nm after incubation. The obtained data made it

possible to determine the methanolic extract's flavonoid content, which was then expressed in terms of quercetin equivalents (mg/QE.) and compared to the quercetin standard. All experiments were run in triplicates.

2.9. Determination of total phenolic content

Total phenolic content (TPC) of *A. klaineana* methanolic stem bark extracts was measured using a slightly modified Folin–Ciocalteau technique [39,40]. Initially, a test concentration of 10 mg/mL for the extracts was obtained by mixing 10 μL of the sample with 790 μL of distilled water. Next, 50 μL of Folin-Ciocalteau reagent were added to these dilutions and thoroughly mixed. An additional 150 μL of a 7% Na_2CO_3 solution was added after an 8-min dark incubation, and this was followed by a 2-h dark incubation at room temperature. Using a Synergy HT Biotek microplate reader, the mixtures' absorbance was calculated at 750 nm. Gallic acid (GA) was used as a reference. The TPC was calculated using its calibration curve, and the results were expressed in milligrams of GA equivalents per gram of dry mass (mg GAE/g DM). The experiments were performed in triplicates.

2.10. Cytotoxicity activity

An MTT assay was used to assess the cytotoxic effects of *A. klaineana* methanolic stem bark extracts (methanol, hexane, chloroform, and ethyl acetate) against mammalian cells. The cell lines used in the study were HepG2 and PNT2 (ATCC, Rockville, USA). HepG2 and PNT2 were all grown in DMEM and RPMI growth media respectively. After cells had reached a confluence of more than 80% and were assessed to be viable using trypan blue staining, these cells were placed at a density of 5×10^3 cells/well in a 96-well plate and incubated for 24 h at 37 °C in 5% CO_2 [41]. After the first incubation, the old medium was changed, and the cells were incubated for a further 24 h in conditions that were comparable while being exposed to a range of plant extract concentrations (from 0 to 1000 μg/mL). For this investigation, curcumin served as the reference control. Following a second 24-h period, the previous medium was changed once more. After the addition of 10 μL of an MTT solution (5 mg/mL in phosphate buffer) to each well, the plate was incubated for 4 h at 37 °C in a 5% CO_2 environment. After removing the medium, any formazan crystals that remained were dissolved in each well using 100 μL of DMSO for 30 min at 37 °C in a 5% CO_2 atmosphere. The color intensity of the dissolved formazan, which appears as a purple tint, was then measured at a wavelength of 570 nm using a Synergy HT Biotek microplate reader. Using GraphPad Prism Software's version 9 (GraphPad software, Boston, MA, U.S.A), the data was examined to find the IC_{50} values. IC_{50} values were calculated from a non-linear regression model using the Hill function. All the experiments were performed in triplicates.

2.11. Determination of antioxidant activity

2.11.1. FRAP assay

Following the directions provided by the kit, the antioxidant capacity of the *A. klaineana* methanolic extract—more especially, its ferric-reducing potential—was assessed using an assay kit (MAK369, Sigma-Aldrich, USA). *A. klaineana* methanolic extract (10 μL), 4 μL FRAP reference control (2 mM), 6 μL FRAP buffer (final concentrations ranging from 0 to 20 nmol/well in steps of 4 nmol/well), 152 μL of FRAP assay medium, 19 μL of $FeCl_3$ solution, and 19 μL of FRAP indicator were all combined to create the mixture that was prepared in 96-well plates. After that, this mixture was left to react at 37 °C for an hour. Then, using a Synergy HT Biotek microplate reader, the resultant absorbance was measured at 594 nm. In order to calculate the decreased ferrous ions from the sample's absorbance, a standard curve was created using ferrous ions as a comparison point. The following formula was used to determine the samples' ferrous ion equivalent concentration:

$$\mathrm{FRAP} = \frac{\boldsymbol{B\ xD}}{\boldsymbol{V}}$$

Sample FRAP, also known as mM Ferrous Equivalent (nmol/μL or mM Fe^{2+} equivalents), is calculated as follows: B═(B x D)/V, where D is the sample's dilution ratio, V is the volume of the sample added to the reaction well (in μL), and B is the amount of ferrous ammonium sulphate inferred from the standard curve (in nmol). All the experiments were run in triplicates.

2.11.2. DPPH assay

A modified DPPH method was used to assess the methanolic extract of *A. klaineana*'s capacity to scavenge free radicals [40]. A range of extract dilutions, from 0 to 5 mg/mL, were mixed with a methanolic DPPH solution (0.5 mM). Using an Austrian Synergy HT Biotek plate reader, the mixture's colour shift was detected at 517 nm after 20 min of interaction at room temperature.

The proportion of antioxidant activity was determined using the formula:

$$\%\ \text{Antioxidant activity} = [(A_0 - A_1)/A_0 \times 100]$$

In this case, A_0 denotes the absorbance of the control (methanol), and A_1 denotes the absorbance in the presence of DPPH extract. The comparable standard was ascorbic acid. There were three runs of these tests. We also found the EC_{50}, or the concentration at which the extract exhibited 50% scavenging activity. All the experiments were conducted in triplicates.

2.12. In-vitro anti-trypanosomal activity

2.12.1. Culturing of Trypanosoma brucei brucei

T. b. brucei (GUTat 3.1 strain)(Tokyo Medical & Dental University, Tokyo, Japan) parasites were cultured in Hirumi's Modified Iscove's Medium-9 (HMI-9) supplemented with 1% penicillin-streptomycin and 10% heat-inactivated fetal bovine serum (Gibco), incubated at 37 °C and 5% CO_2 according to previously established protocol with slight modification [42]. Parasite growth was observed daily using the Neubauer's chamber until they reach a level of confluence (usually at 10^6 parasites/mL).

The stem bark extracts (methanol, hexane, chloroform, and ethyl acetate) of *A. klaineana* were tested for anti-trypanosomal activity using the Alamar blue assay against *T. b. brucei* parasites. Stock solutions (20 mg/mL) of extracts and purified compounds are prepared in dimethyl sulfoxide (DMSO) and filter-sterilized through a 0.2 μm Millipore filters (Nylon syringe filter, Macherey-Nagel, Fisher Scientific, Dublin, Ireland). A stock concentration of 20 mg/mL (100% DMSO) of compounds/extracts were diluted with culture media to a working concentration of 2000 μg/mL (0.01% DMSO). A serial dilution of 1:2 concentrations of the compounds/extracts (200–0.78125 μg/mL) was prepared in 96 well plates. Trypanosome parasites cultured overnight to a density of 1×10^6 cells/mL were diluted to a concentration of 4×10^3 parasites/mL with HMI-9 media used for the assay. Test wells were initially seeded with 50 μL of plant extracts/compounds, followed by 50 μL of parasite suspension. The final concentration of DMSO in the preparation was below 1%. Each sample was done in triplicates. The positive control used was Diminazene aceturate (DA), a commercially antitrypanosomal drug, negative controls were growth media only. The plates were incubated for 48 h at 37 °C in 5% CO_2, then 10% Alamar blue dye was added, and the parasites were incubated for another 4 h in darkness. After 4 h incubation, the plate was read for absorbance at 540 nm using the wavelength of 530 nm and an emission wavelength of 590 nm, fluorescence was measured using a Varioskan Lux Elisa microplate reader (Thermo Fischer Scientific, USA). All the experiments were run in triplicates. Data were analyzed using GraphPad prism version 9(GraphPad software, Boston, MA, U.S.A). The IC_{50} values (concentration of extracts or compounds that inhibits 50% of growth) were determined. The IC_{50} reported for the extracts are the averages from three independent experiments. IC_{50} values were calculated from a non-linear regression model using the Hill function.

2.13. In silico approach

The Simplified Molecular Input Line Entry System (smiles) of compounds present in the stem bark of *A. klaineana* were retrieved from PubChem [43] and fed into SwissADME [44] and Datawarrior [45] to evaluate the drug-likeness and pharmacokinetic properties of the compounds. This was to ensure that only drug-like compounds are utilized for the downstream activities. The smiles of the drug-like compounds were then converted to AutoDock files (pdbqt) after being minimized employing the universal force field.

The 3D structure of TR and UDP were retrieved from the protein data bank [46] with PDB ID 6OEX and 1GY8 solved to a resolution of 2.10 Å and 2.00 Å respectively. The structures were visualized using PyMOL version 2.5.0 [47], removing any bound ligands and water molecules. Leveraging on GROMACS 2018 [48,49], the energy of the proteins was minimized employing the OPLS/AA force field [50]. Prior to the energy minimization, the system was solvated with NaCl of concentration 0.15 M in a cubic simulation box with a gap of 1 nm from the edges [49].

To identify compounds the binding affinity of the drug-like compounds against TR and UDP, the compounds were virtually screened against TR and UDP employing AutoDock Vina version 1.2.1 [51]. Previous studies have shown that AutoDock Vina can distinguish between active and non-active binders with an AUC of ROC curves ranging between 0.7 and 0.99. AutoDock Vina has also been shown to successfully predict the binding pose of ligands in the binding pocket of proteins [52,53–55].

To characterize the mechanism of binding of the compounds docked against TR and UDP, Discovery studio Visualizer v21.1.0.20298 [56] was utilized. Both the 3D and the 2D visualization of the protein-ligand interaction was performed revealing the amino acid residues the compounds interacted with.

3. Results and discussion

3.1. Preliminary phytochemical analysis of methanolic stem bark extracts of A. klaineana

Medicinal plants are rich in essential phytochemicals which are crucial to their biological activities [57]. Plant-derived metabolites

Table 1
Preliminary Phytochemical screening of *A. klaineana* stem bark methanolic crude extract.

Phytochemicals	*Anopyxis klaineana* (AK)
Alkaloids	+
Saponins	+
Flavonoids	+
Phenols	+
Steroids	–
Terpenoids	+
Tannins	+

KEY: (+) Present, (−) Absent.

have several biological activities and health benefits including anticancer, anti-inflammatory, antioxidant, antimalarial and antimicrobial properties [41,58–61]. In this study, the methanolic stem bark crude extracts of *A. klaineana* was subjected to preliminary phytochemical analysis to identify the various secondary metabolites present using qualitative methods [62,37]. Results from our analysis indicated the presence of a variety of secondary metabolites such as alkaloids, saponins, flavonoids, phenols, terpenoids and tannins in the stem bark of *A. klaineana* as depicted in Table 1. Alkaloids are diverse phytochemicals with wide different biological activities including antimicrobial, antiviral, antimalarial, anti-inflammatory and antioxidant [63,64]. Alkaloids have been demonstrated to have anti-trypanosomal properties [65]. Polyphenols are natural phytocompounds frequently present in plants. They include flavonoids and phenolics and well known for their health benefits and biological properties such as antioxidant, anti-inflammatory, antimicrobial, anticancer and antiparasitic [66–70]. Many studies have reported polyphenols as antitrypanosomal agents [71–76]. Tannins are phytochemicals that have been demonstrated to possess anti-inflammatory, antimicrobial, antioxidant, immunomodulatory, and antiparasitic properties [77,78]. Saponins are phytochemicals found in plants and belong to a large family of steroid glycosides and triterpenes [79]. Saponins have demonstrated a wide range of biological properties such as antiviral, anticancer, antioxidant, anti-inflammatory, immunomodulatory effects and antiparasitic [80–82]. Terpenoids are diverse group of phytochemicals commonly found in plants. They have a wide range of therapeutic usage and used for the treatment of many diseases [83]. Terpenoids have been demonstrated to have antitrypanosomal properties [16]. Antitrypanosomal activities observed in this study could be attributed to the major phytochemical constituents present in the extracts.

3.2. FITR analysis of *A. klaineana* stem bark methanolic crude extract

FITR analysis showed various functional groups present in the stem bark methanolic crude extract of *A. klaineana* as depicted in Fig. 1. The peak, functional group and chemical class obtained from the absorption spectra are represented in Table S1. Peak 1 at 3277.39 cm^{-1} indicates OH functional group which corresponds to alcohol and phenol compounds. Peak 2 detected at 2927.97 cm^{-1}, represents C–H stretching alkane compounds. The observed peak 3 at 1604.96 cm^{-1}, indicates C═C stretch of alkene compound. In between the peak range 1600-1300, two peaks (4&5) were observed at 1518.81 cm^{-1} and 1441.56 cm^{-1}, indicating N–O stretching of nitro compounds and C–H/OH bending of alkane, alcohol and carboxylic acid respectively. Peak 6 at 1032.50 cm^{-1} represents C–O stretching of Alcohols, carboxylic acids, esters, and ethers. The functional groups identified (peak1,2,3,4,5 &6) indicates the main phytochemicals such as alkaloids, terpenoids and polyphenols present in the methanolic crude extract of stem bark of *A. klaineana*. These phytochemicals have been reported to have medicinal properties such as antioxidant and antiprotozoal activities.

3.3. LC-MS analysis of stem bark of *A. klaineana* methanolic extract

In this present study, the methanolic crude stem bark extract of *A. klaineana* was subjected to phytochemical profiling using LC-ESI-QTOF-MS analysis to identify potential phytochemicals present. Twenty-four (24) bioactive compounds were tentatively identified from their mass data and MS spectra using Agilent LC-MS Qualitative Software (Mass Hunter) and Personal Compound Database and Library (PCDL) as illustrated in Fig. 2 and Table S2. Additional public databases such as; PubChem [43], ChemSpider [84] and Phenol-Explorer [85] were included. The chromatogram is depicted in Fig. 2, while the potential bioactive compounds with their molecular formula, retention time (RT), theoretical mass (*m/z*), observed mass (*m/z*), mass error (ppm) and chemical category are presented in Table S2. The compounds identified belong to different chemical classes including alkaloids, flavonoid, terpenoids,

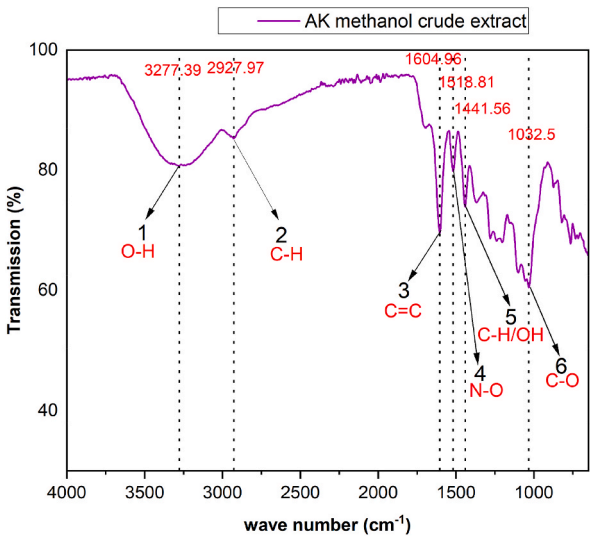

Fig. 1. FTIR spectrum analysis of stem bark methanolic crude extract of *A. klaineana*.

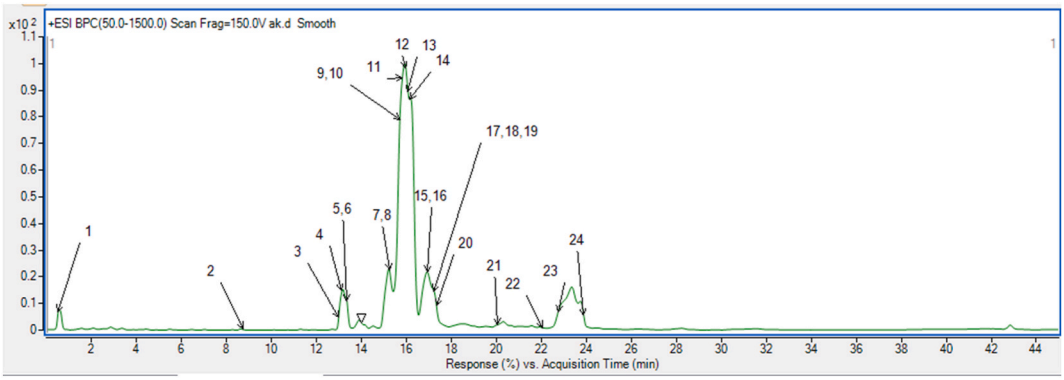

Fig. 2. The base peak chromatogram (BPC) of *A. klaineana* stem bark methanolic extract in positive ionization mode.

lignan, phenol, fatty acids, glycoside, Vitamin, steroid saponin, Fatty alcohol, Amine, Pyrimidine, Lactones, Steroid and Sterol. The majority of the compounds identified were terpenoids and lactones. The terpenoids which include: Carbenoxolone, Dihydro-7-Desacetyldeoxygedunin, Fusidic acid and Acinospesigenin A. Plant terpenoids have been shown to have antitrypanosomal activities against Trypanosoma parasites [16]. Dihydro-7-Desacetyldeoxygedunin is structurally similar to two natural limonoids (7-deacetylgedunin and 7-deacetyl-7-oxogedunin) isolated from the roots of Pseudocedrela kotschyi, have been reported to have antitrypanosomal activities [86]. Helvolic acid which is an analog of Fusidic acid has been shown to possess antitrypanosomal activity against *T. b. brucei* parasite with IC_{50} value of 5.08 μg/mL [87]. Lactones are naturally occurring diverse compounds with wide range of biological properties. Research studies have demonstrated that they possess antitrypanosomal activities [88]. Lactones detected in the methanolic extracts include Tylvalosin, Lucimycin, Nemadectin, and Bryostatin 1.

Structurally similar compounds of Lucimycin and Bryostatin 1 have been reported show potent antitrypanosomal activities against *T. b. brucei* and *T. b. rhodesiense* [89]. These potentially bioactive compounds could be attributed to their therapeutic potential of the extracts of *A. klaineana*.

3.4. Determination of the total phenolic and total flavonoid content of A. klaineana stem bark methanolic crude extract

Polyphenols such as flavonoids and phenolics are natural phytocompounds that present in plants. They are generally known for their health benefits and biological properties [90]. Phenolic and flavonoid compounds are major antioxidant components that have the essential role of stabilizing free radicals due to their ability to donate hydrogen atoms [91]. In addition the presence of phenolics and flavonoids may interrupt the chain reaction of reactive oxygen species in cellular mechanisms, thus protect the human body from reactive oxygen species damages [92]. It is therefore essential to determine the presence of these compounds in plants. The total phenolic and flavonoid contents of the methanolic stem bark extract of extract of *A. klaineana* were determined using Folin–Ciocalteau and the aluminium chloride ($AlCl_3$) colorimetric method respectively. The results observed for total phenolic content (TPC) and total flavonoid content (TFC) of *A. klaineana* stem bark methanolic extract were 17.3 ± 0.3 GAE and 290.4 ± 0.2 mg/g QE respectively as depicted in Table 2. The total phenolic content (TPC) was expressed in mg gallic acid equivalent per 100g using the standard calibration curve ($y = 0.9301x-0.0041$, $R^2 = 0.9888$) Fig. 2SA. The total flavonoid content (TFC) was expressed in mg quercetin equivalent per 100g using the standard calibration curve ($y = 100.09x+0.111$, $R^2 = 0.9666$) Fig. 2SB. The presence of phenolic and flavonoid compounds in the methanolic extracts could attribute to its antioxidant activities observed in this study.

Table 2
Antioxidant activity, total phenolic content, and total flavonoid content of *A. klaineana* methanolic crude extract.

Assay	*A. klaineana* extract	Standard (Ascorbic Acid)
DPPH[a]	0.043 ± 0.003	0.0341 ± 0.006
FRAP[b]	35.1 ± 0.5	NA
TPC[c]	17.3 ± 0.3	NA
TFC[d]	290.4 ± 0.2	NA

Data are represented as means and standard error of the mean for a triplicate experiment.
FRAP-ferric reducing antioxidant power, **DPPH**- 2,2-diphenyl-1-picryl-hydrazyl-hydrate, **TPC**-Total Phenolic Content, **TFC**-Total Flavonoid Content.
[a] -expressed as EC_{50} in mg/ml of extract.
[b] -expressed as ferrous equivalent in mM.
[c] -expressed in mg gallic acid equivalent per 100g, and.
[d] -expressed in mg quercetin equivalent per 100g. **NA**-not applicable.

3.5. The antioxidant activity of A. klaineana methanolic crude extract

Antioxidants are molecules that protects or prevent biological tissues from oxidative stress or damage by stabilizing free radicals or reactive oxygen species (ROS) during cell metabolism [93]. Research have shown that antioxidants plays a significant role in maintaining human health and prevention of diseases due to their ability to reduce oxidative stress [94,95]. Medicinal plants are known be good source of antioxidants [96,97] and many studies have been reported [98–101]. ROS contributes to initiation and progression of several diseases including protozoal diseases [102–104]. High levels of ROS helps promotes the replication of trypanosome parasite, which have serious implications on the stages of the disease [105–107], Thus antioxidants used as adjuvant therapy could help reduce oxidative damage in the host's tissues by neutralizing free radicals. Having an antitrypanosomal compounds with antioxidant properties will be beneficial in condition such as trypanosomiasis, which produces ROS in response to the parasite infection [108], hence the stem bark methanolic crude extracts of *A. klaineana* were assessed for their antioxidant properties using FRAP and DPPH assays. These assays are simple and widely used in the estimation of antioxidant activities [109,110]. The DPPH assay measures the free radical scavenging activity of antioxidants. It uses free radicals as hydrogen donors or free radical scavengers and determined by its ability to decolorize the stable DPPH from purple to yellow or colourless. The degree of colour change is proportional to the concentration and the antioxidant potency. The reading of DPPH free radical scavenging activity was expressed as $EC_{50,}$ thus the half maximal effective concentration that can cause 50% free radical scavenging activity. Our results revealed free radical scavenging activity of EC_{50} value of 0.043 ± 0.03 mg/mL of stem bark extract of *A. klaineana* as compared to the standard ascorbic acid (Table 2). A previous study by Mireku et al. reported the DPPH free radical scavenging properties of compounds isolated from stem bark extract of *A. klaineana,* this confirms the antioxidant potential of this plant [24].

The FRAP assay uses antioxidants as reductants in a redox linked colorimetric reaction to measure the potential samples to reduce Fe^{+3} to Fe^{+2}. In our study, the FRAP antioxidant activity was observed in the methanolic stem bark extract of *A. klaineana* with a ferrous equivalent of 35.1 ± 0.5 mM (Table 2) using the standard calibration curve ($y = 100.09x+0.111$, $R^2 = 0.9666$) Fig. 2SC. The presence of antioxidants in the methanolic crude extracts of *A. klaineana* may have cause a reduction of Fe^{+3} to Fe^{+2} proving its reducing power. Results from our study suggest that the methanolic stem bark extracts of *A. klaineana* have antioxidant potentials and this is likely due to the presence of polyphenols present in the crude extracts. These extracts could therefore be developed as therapeutics in preventing oxidative stress-related diseases.

3.6. Cytotoxicity activities of A. klaineana stem bark extracts

In this study, cytotoxicity activities of stem bark of *A. klaineana* extract (methanol, hexane, chloroform and ethyl acetate) were assessed using MTT assay against HepG2 and PNT2 cell lines. The MTT (3-(4,5-dimethylthiazol-2-yl)-2–5-diphenyltetrazolium bromide) assay is the most widely used method to assess cytotoxicity and cell viability [111]. MTT assay uses a reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to measure cellular metabolic activity as a proxy for cell viability. Viable cells contain mitochondrial enzymes which reduce the MTT reagent to formazan, an insoluble crystalline product with a deep purple colour. The amount of colour produced is directly proportional to the number of viable cells. The darker the solution, the greater the number of viable, metabolically active cells [111]. According to Godwin et al., [112], cytotoxicity of plant extracts are classified as: 1) highly toxic if $CC_{50} \leq 20$ μg/mL, 2) moderately toxic if $CC_{50} = 21–200$ μg/mL, 3) weakly toxic if $CC_{50} =$ 201–500 μg/mL, 4) non-toxic if $CC_{50} \geq 501$ μg/mL. Results from our study, showed that the stem bark methanolic extracts of *A. klaineana* displayed moderately cytotoxic against HepG2 and PNT2 cell lines, with IC_{50} values of 68.0 ± 2.05 and 78.7 ± 2.63 μg/mL respectively as compared to positive control (curcumin) (Table 3). In-addition, the hexane, chloroform and ethyl acetate extracts were not toxic to HepG2 cell.

In reference to Godwin et al.'s cytotoxicity classification [112], our study demonstrated that the *A. klaineana* stem bark methanolic extracts were moderately cytotoxic against HepG2 and PNT2 cell lines, with IC_{50} values of 68.0 ± 2.05 μg/mL and 78.7 ± 2.63 μg/mL, respectively. Comparing these values to the criteria set by Godwin et al., our extracts fell within the moderately toxic range ($CC_{50} =$ 21–200 μg/mL).

Calculating the fold difference between our IC_{50} values and the upper limit of moderately toxic classification (200 μg/mL) provides

Table 3
In-vitro antitrypanosomal and cytotoxicity activities of *A. klaineana* plant extracts.

Extracts	*T. b. brucei* [IC_{50}] μg/mL	HepG2 [CC_{50}] μg/mL	PNT2 [CC_{50}] μg/mL	SI	
				[HepG2]	[PNT2]
AK methanolic extract	21.25 ± 0.755	68.0 ± 2.05	78.7 ± 2.63	3.2	3.7
Hexane extract	4.35 ± 0.166	>100	ND	>22.9	**ND**
Chloroform extract	2.57 ± 0.153	>100	ND	>38.9	**ND**
Ethyl acetate extract	22.92 ± 2.321	>100	ND	>4.4	**ND**
Curcumin	ND	5.3 ± 0.53	5.3 ± 0.53	ND	**ND**
DA	0.01447 ± 0.001	ND	ND	ND	**ND**

Data are represented as means and standard error of the mean for a triplicate experiment. The selectivity index **(SI)** is a ratio between the CC_{50} value of the human cell lines (HepG2 or PNT2) to the IC_{50} value of the *T. b. brucei*. CC_{50} – 50% cytotoxicity concentration, IC_{50} – 50% inhibitory concentration, **ND**-Not determined, **DA**-Diminazene aceturate (antitrypanosomal drug). DA and curcumin were used as positive controls.

a perspective on the relative cytotoxicity. For instance, the fold difference for HepG2 cells would be 200 μg/mL/68.0 μg/mL ≈ 2.94, indicating that our extracts were approximately 2.94 times less toxic than the upper limit for moderately toxic substances. Likewise, PNT-2 cells exhibited a toxicity level approximately 2.54 times lower than the upper limit set for moderately toxic substances.

This fold difference metric offers a quantitative measure of how our extracts compare to the established toxicity categories, providing valuable insights into the safety profile of *A. klaineana* extracts in relation to the defined toxicity thresholds.

3.7. Anti-trypanosomal activity of A. klaineana extracts

The anti-trypanosomal activities of stem bark of *A. klaineana* extracts (methanol, hexane, chloroform and ethyl acetate) were evaluated against *T. b. brucei* parasite using Alamar blue cell viability assay after 48 h incubation. Results from our study showed that A. *klaineana* extracts (AK-Methanol, A-Hexane, A-Chloroform and A-Ethyl Acetate) exhibited potential anti-trypanosomal activities against the *T. b. brucei* parasites with IC_{50}values of 21.25 ± 0.755, 4.35 ± 0.166, 2.57 ± 0.153 and 22.92 ± 2.321 μg/mL respectively as compared to the positive control (Diminazene aceturate) (Table 3)(Fig. 3 (A) –(E).

According to Osorio et al. [113], *invitro* anti-trypanosomal activity of plant extracts are categorised as: 1. highly active $IC_{50} < 10$ μg/mL, 2. active $10 < IC_{50} < 50$ μg/mL, 3. moderately active $50 < IC_{50} < 100$ μg/mL, and 4. inactive $IC_{50} > 100$ μg/mL. Thus, the chloroform and hexane extracts were classified as highly active, whiles methanol and ethyl acetate extracts were classified as active. The chloroform extract displayed the strongest antitrypanosomal activity against trypanosome parasites.

The selectivity index of the extracts was determined using the two mammalian cells (HepG2 or PNT2). Selective index (S.I) is defined as the ratio of the CC_{50} of mammalian cells and IC_{50} for trypanosome parasites. The S.I of methanolic crude extract were for 3.2 and 3.7 HepG2 and PNT2 respectively. The S.I for the hexane, chloroform and ethyl acetate extracts were >22.9, >38.9 and >4.4 respectively for HepG2 cell line. The higher the S.I., the safer and more promising the extract due to its selectiveness to the trypanosome parasites.

To the best of our knowledge, this is the first report of antitrypanosomal activities of *A. klaineana* extracts. Our current findings demonstrated that the stem bark of *A. klaineana* extracts may contain useful antiprotozoal leads and provides the scientific basis for the traditional use of this plant to treat protozoan diseases.

3.8. In silico approach

3.8.1. Pharmacokinetic studies of bioactive compounds identified in A. klaineana stem bark methanolic crude extract

To assess the drug-likeness and pharmacokinetic properties of the compounds, Lipinski's Ghose, Veber, Egan and Muegge rules as well as GI absorption and Blood Brain Barrier (BBB) permeability were employed. These rules are analytical approach for predicting drug-likeness stating that molecules had molecular weight (MW ≤ 500 Da), high lipophilicity expressed as log P (log P ≤ 5), hydrogen bond donors (HBDs ≤5), hydrogen bond acceptors (HBAs ≤10) topological polar surface area (TPSA ≤140), number of rotatable bond

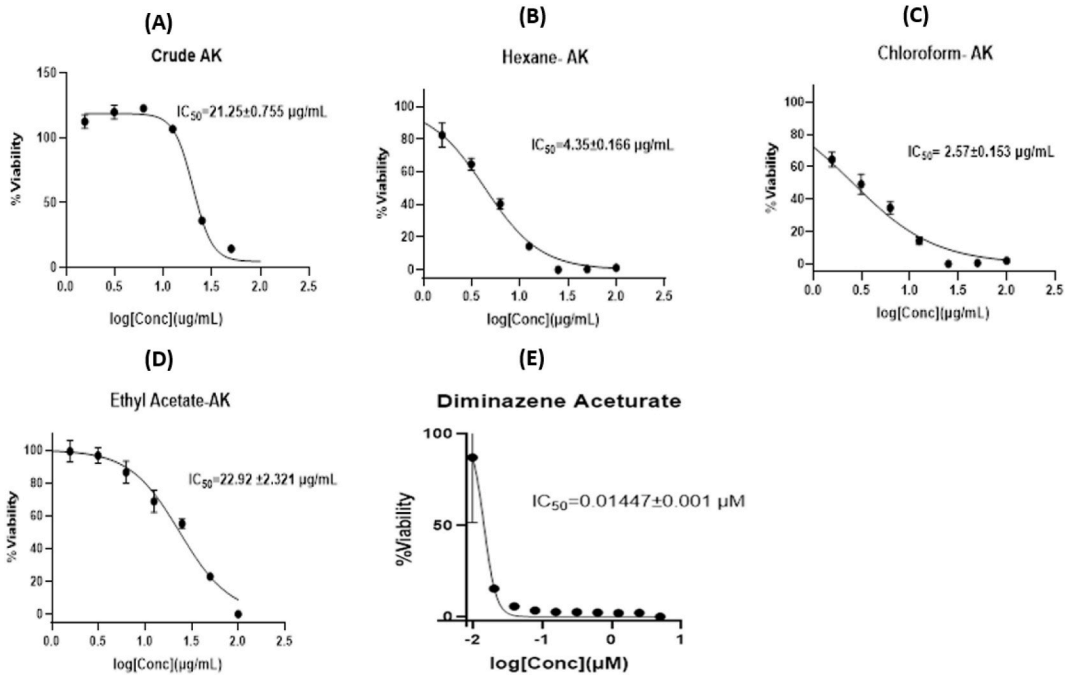

Fig. 3. Dose-response curves of antitrypanosomal activity of methanolic crude extract (A), hexane extract (B), Chloroform extract (C), Ethyl Acetate extract (D) and Diminazene Aceturate (E).

(R. bond ≤10) and number of rings (Rings ≤7) with good absorption or permeation across the cell membrane [44]. Out of the 24 compounds, only 5 compounds did not violate any of the rules and were also predicted to have a high GI absorption with Dimethylmatairesinol and risperidone predicted to be BBB permeant (Table S3).

3.8.2. Molecular docking of compounds

The use of *in-silico* methods including molecular docking is playing an increasing important role in drug discovery, which are necessary for cost effective identification of promising drug candidates [114]. Several studies have reported the use of this methods to discover compounds against Neglected Tropical Diseases [52,115–118].

Molecular docking is a molecular modelling technique that predict the binding affinity between protein target and a ligand [119]. This interaction forms a supramolecular complex that plays a major role in the dynamics of the protein target, thereby enhancing or inhibiting its biological function [120].

To dissect the mechanism action of antitrypanosomal activity of the stem bark methanolic extract of *A. klaineana* using *in-silico* approach, the drug-like compounds identified were virtually screened against the active sites of TR and UDP. This was to identify the binding affinity between the compounds and the targets and the binding pose of the compounds in the binding pocket of the targets [30].

Both TR and UDP are crucial enzymes that play significant role in the trypanosome parasite's growth, survival and its virulence. Hence, making them an avenue for potential therapeutic targets [121]. UDP is a key enzyme involved in galactose metabolism of trypanosome parasite. Galactose is a component of surface glycoproteins that plays role in the evasion of the parasite [122]. TR is an essential enzyme involve in major defence mechanism against oxidative stress for trypanosome parasites [123].

Results from molecular docking studies showed that, Risperidone, dihydro-7-Desacetyldeoxygedunin, 6beta-Hydroxytriamcinolone acetonide, ranolazine and Dimethylmatairesinol had binding energies of −10.4, −8.7, −8.4, −7.9 and −7.1 kcal/mol against TR and −10.8, −8.4, −7.6, −8.4 and −8.1 kcal/mol against UDP **(Table 4).** All the compounds had significantly high binding affinity (<- 7.0 kcal/mol) as it has been reported than −7.0 kcal/mol distinguish between putative and non-putative binders [124]. The binding pattern of compounds with TR and UDP may hinder the substrate accessibility and its subsequent inhibition as shown in Fig. 4 (A) and **(D)** for TR-risperidone complex, Fig. 4 (B) and **(E)** for TR-ranolazine complex and Fig. 4 (C) and **(F)** for TR-dimethylmatairesinol complex while Fig. 5 (A) and **(D)** for UDP-risperidone complex, Fig. 5 (B) and **(E)** for UDP-dimethylmatairesinol complex and Fig. 5 (C) and **(F)** for UDP-ranolazine complex.

Gly7, Gly10, Ile12, Asp75, Ala100, Asn117, Ser141, Ser142, Ala143, Tyr173, Asn202, His221 and Arg268 are the active site residues of UDP with the UDP sugar interacting with Ser142, Asn202, His221, Ser143, Tyr173, Lys117 and Arg268 [32]. Leu102 has also been reported to play I crucial role in the stability of the intermediate of UDP, implying that Leu102 is a good residue to target when designing inhibitors of UDP [32]. Among the active site residues, risperidone interacted with Ser141, Ile12 and His221, ranolazine interacted with Ile12 and Dimethylmatairesinol interacted with Gly10 and Ile12 **(Table 4).** Risperidone, ranolazine and Dimethylmatairesinol also interacted Leu102 (Table 4 and Fig. 5(A)–(F)).

Leu17, Trp21, Cys52, Cys57, Tyr110, Met113, Glu18, Ser109 and Ala343 are the active site residues of TR and they play critical role in the catalytic activity of TR [34,35]. Risperidone, ranolazine and Dimethylmatairesinol interacted with Cys57 (Table 4 and Fig. 4 (A)–(F)). For a compound to inhibit a target protein, it must interact with the critical residues of the protein [125] hence, risperidone, ranolazine and Dimethylmatairesinol might inhibit both TR and UDP.

Results from our *in-silico* studies suggest that the bioactive compounds possibly inhibited the key therapeutic targets of *T. brucei,*

Table 4
Binding energy of compounds virtually screened against trypanothione reductase and uridine diphosphate galactose 4′-epimerase.

	Protein Target	COMPOUND	CID	BINDING ENERGY kcal/mol	INTERACTED RESIDUES
1	***Trypanothione reductase***	Risperidone	5073	−10.4	Phe198, Ile199, Lys60, Cys57, Leu334, Phe367, Gln439, Pro435, Ala365
		Dihydro-7-Desacetyldeoxygedunin	6708643	−8.7	Phe198, Leu332, Val362, Arg287
		6 beta-Hydroxytriamcinolone acetonide	29982180	−8.4	Thr374, Ser364, Val362, Phe230, Phe198
		Ranolazine	56959	−7.9	Lys60, Phe198, Cys57, GLY56, Ile199, Arg287, Ala365, Ala363
		Dimethylmatairesinol	1286	−7.1	Ala365, Cys57, Asp327, Leu334, Phe198, Met333, Ile199
2	***Uridine diphosphate galactose 4′-epimerase***	Risperidone	5073	−10.8	Ala103, Gly36, Leu102, Met98, Lys177, Ala100, Ser140, Tyr200, Ser141, Ile12, Tyr11, His221
		Dihydro-7-Desacetyldeoxygedunin	6708643	−8.4	Asp42, His376, Arg18
		Ranolazine	56959	−8.4	His215, Leu102, Ala203, Ile12, Gly36, Tyr380, Met98, Tyr200, His43
		Dimethylmatairesinol	1286	−8.1	His43, Ala9, His215, Tyr380, Gly10, Ala100, Met98, Ile12, Leu102, Ser219
		6 beta-Hydroxytriamcinolone acetonide	29982180	−7.6	Phe101, Ser219, Gly36, Val35

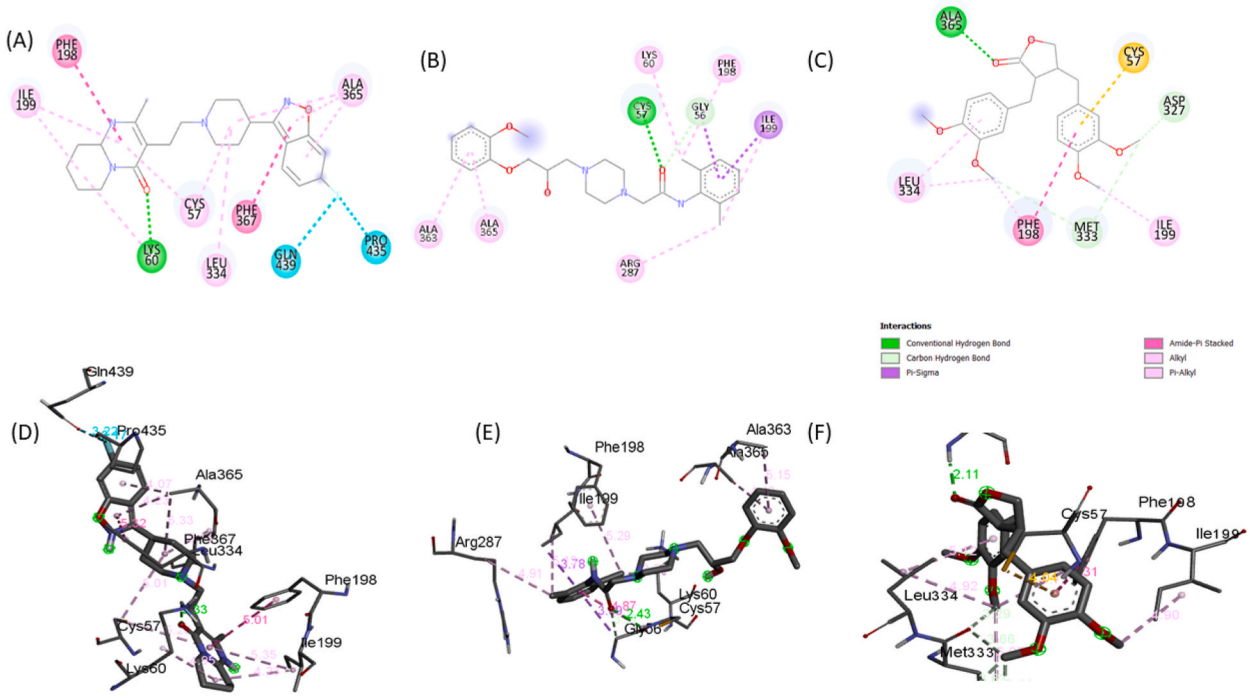

Fig. 4. Protein-ligand interaction of (A) and (D) TR - risperidone complex, (B) and (E) TR - ranolazine complex and (C) and (F) TR – dimethylmatairesinol complex.

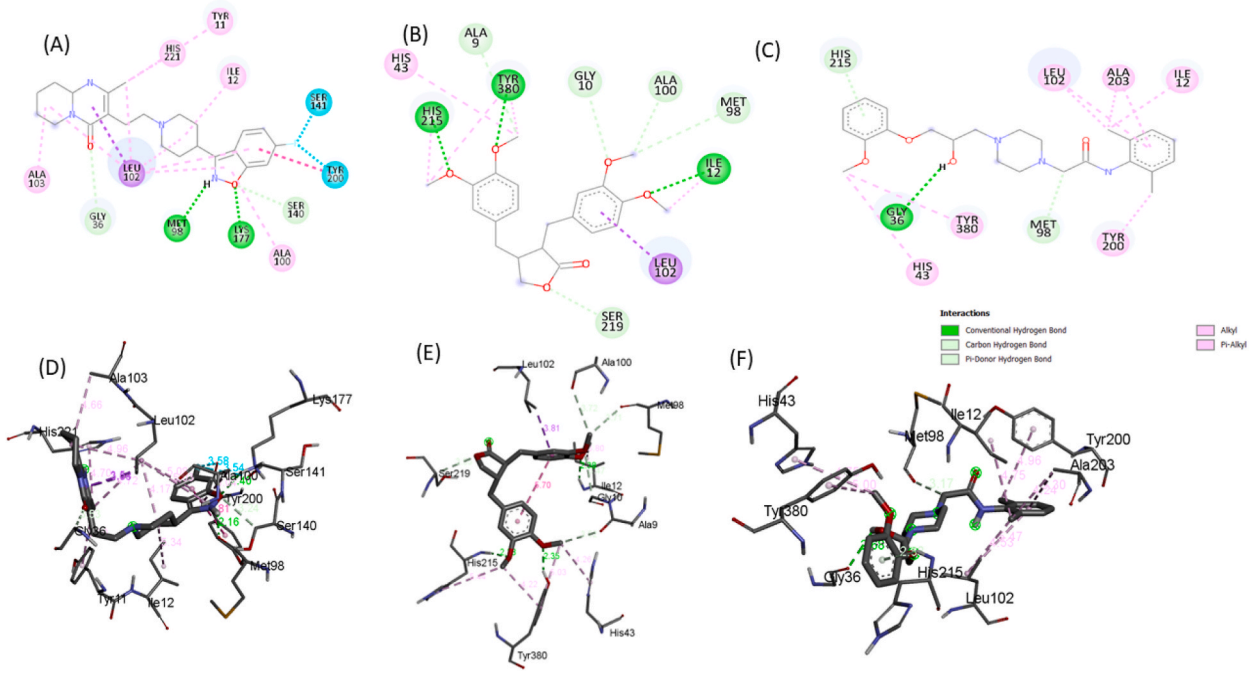

Fig. 5. Protein-ligand interaction of (A) and (D) UDP - risperidone complex, (B) and (E) UDP - dimethylmatairesinol complex and (C) and (F) UDP - ranolazine complex.

which plays role in their growth, survival and virulence. Again, the *in-vitro* anti-trypanosomal activities observed in this study, could be attributed to the inhibition of TR and UDP of trypanosome parasite.

3.8.3. Biological activity exploration of hits

Prediction Activity of Substance Spectra (PASS) was liaised to predict the antiprotozoal activity of the hits [126]. PASS employs a Quantitative Structure Activity Relationship (QSAR) approach to predict the activity of a compound leveraging on known drugs and clinical trials drugs. With a probable of active (Pa) values and probable of inactive (Pi) values, the activity of compounds are estimated with compounds having Pa values greater than Pi values are worthy of further exploration [126]. Dihydro-7-Desacetyldeoxygedunin, Dimethylmatairesinol and ranolazine were predicted to be antiprotozoal with Pa values of 0.684, 0.537 and 0.261 while having Pi values of 0.010, 0.020 and 0.055 correspondingly. This corroborates our findings that the bark methanol extract of *A. klaineana possess antitrypanosomal activity.*

4. Conclusion

Our findings showed that the stem bark extracts of *A. klaineana* demonstrated potential antitrypanosomal activities through *in-vitro* and *in-silico* studies. According to our knowledge this is the first report of anti-trypanosomal activities of the stem bark extracts of *A. klaineana.* Moreover, Significant antioxidant potential was also observed in the extracts of stem bark of *A. klaineana,* indicating a multifaceted therapeutic potential. LC-MS analysis identified 24 potentially bioactive compounds with 5 being druglike. In the *in-silico* studies, Risperidone, Ranolazine, Dihydro-7-Desacetyldeoxygedunin, 6 beta-Hydroxytriamcinolone acetonide and Dimethylmatairesinol were identified as novel potential inhibitors of TR and UDP with binding affinities of −10.4, −7.9, −8.7, −8.4 and −7.1 kcal/mol respectively against TR and −10.8, −8.4, −8.4, −7.6 and −8.1 respectively against UDP. These compounds could serve as candidates for the design of novel antitrypanosomal therapeutics and they should further be experimentally investigated to determine their potential efficacy.

However, as we progress, it is crucial to address several notable limitations in our study on the anti-trypanosomal effects. Firstly, the strain specificity observed in our results highlights the need for more extensive investigations across various Trypanosoma species and strains to ensure the generalizability of these findings. While the identified potential bioactive compounds show promise, their transformation into effective anti-trypanosomal drugs necessitates rigorous evaluation, including safety assessments, optimal dosage determination, and comprehensive pharmacological studies.

Again, the study did not quantify the compounds identified in *A. klaineana* stem extract exhibiting antiparasitic activity. However, future work should prioritize quantifying these compounds to better understand their pharmacological properties.

Given these considerations, it is vital to underscore the immediate need for sustained research efforts. Further exploration into the *in vivo* effectiveness and safety profiles of these extracts and their bioactive constituents is essential to establish their therapeutic potential against trypanosomiasis. This study provides a strong foundation, highlighting the need for ongoing research, which will be instrumental in the development of novel anti-trypanosomal agents. Finally, the results of our study contribute to the validation of ethnomedicinal use of *A. klaineana* for treatment of parasitic diseases.

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Data availability statement

All data generated and analyzed during this study are included in this manuscript and the supplementary data. Data associated with this study has not been deposited into publicly available repository, However data will be made available upon request.

CRediT authorship contribution statement

Latif Adams: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Dorcas Obiri Yeboah:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology. **Michael Afiadenyo:** Writing – original draft, Visualization, Validation, Data curation. **Sherif Hamidu:** Methodology, Investigation, Formal analysis. **Abigail Aning:** Methodology, Investigation. **Ebenezer Ehun:** Methodology, Investigation. **Katie Shiels:** Methodology, Investigation. **Akanksha Joshi:** Methodology, Investigation. **Maxwell Mamfe Sakyimah:** Methodology, Investigation. **Kwadwo Asamoah Kusi:** Writing – review & editing, Validation, Supervision, Methodology. **Irene Ayi:** Validation, Supervision, Methodology. **Michelle Mckeon Bennett:** Writing – review & editing, Supervision, Project administration. **Siobhan Moane:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition.

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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Zuputo, a high-performance computer system for biocomputing at the WACCBIP, university of Ghana.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28025.

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