In-Vivo and In-Silico Evidence of Antitrypanocidal Activities of Selected Plants from Asteraceae Family against Trypanosoma brucei brucei

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Abstract: The menace of animal African trypanosomiasis (AAT) has increased in sub-Saharan Africa despite countless interventions through chemotherapy and health policies. The study evaluated the in-vivo antitrypanosomal activities of five plant species from the Asteraceae family and further subjected some previously isolated compounds from the active plants to molecular docking on an identified T. brucei target. The extracts of the aerial parts of Tithonia diversifolia, Chromolaena odorata, Aspilia africana, Vernonia glaberrima, and Synedrella nodiflora were subjected to in-vivo antitrypanosomal activity against T. b. brucei-infected mice by monitoring the parasitemia and packed cell volume (PCV) at intervals. Some isolated compounds were docked to a co-crystallized ornithine decarboxylase (ODC) enzyme target, and the binding energies were compared. All the extracts (200-600 mg/kg) caused a significant (p < 0.05) decline in the parasitemia level compared with the untreated group. Both T. diversifolia (200-600 mg/kg) and V. glaberrima (400-600 mg/kg) caused total clearance of the parasite from the bloodstream of infected mice by the 17th day. Similarly, except for S. nodiflora and C. odorata, all other tested extracts restored the PCV of the infected mice to the baseline value (> 40%). Woodhousin from T. diversifolia and apigenin from V. glaberrima interacted maximally with the ODC of T. b. brucei. The study has established the basis for using the selected plants in the management of AAT in folk medicine and further provided insights into the potential of the plant species to provide new bioactive lead compounds.

Keywords: Asteraceae, Trypanosoma brucei; ethnomedicine; ornithine decarboxylase; docking.

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1. Introduction

Animal African trypanosomiasis (AAT), caused by T. brucei, T. congolense, and T. vivax, is an insect-borne parasitic disease that affects livestock in sub-Saharan Africa [1]. Several preventive and treatment strategies have been deployed to overcome the billions of dollars loss to AAT [2]. These strategies have resulted in slow progress in eradicating AAT compared with human African trypanosomiasis (HAT). At the moment, there is no vaccine against AAT, and sustained control of insect vectors is still difficult. Available treatments are antiquated and hampered by toxicity, limited diagnosis, drug resistance, high cost, and misuse [3,4]. These are key factors in discovering and designing new trypanocides for the management of AAT [3].
Plants have inspired the discovery of many potent trypanocides and have shown promising activities in several cases [5,6]. Five medicinal plants species from the Asteraceae family used in folk medicine across sub-Saharan Africa and beyond were selected for this study: *Tithonia diversifolia* (Hemsl.) A. Gray, *Chromolaena odorata* (L.) R.M. King & H. Rob, *Aspilia Africana* (Pers.) C.D. Adams, *Vernonia glaberrima* (Welw. & Ex O. Hoffm.) and *Synedrella nodiflora* (L.) Gaertn. Despite the usefulness of the aerial parts of these plants for various purposes in folk medicine [7-14], there have been no reported *in-vivo* antitrypanosomal activity.

Isolation of bioactive compounds from plants takes a lot of time and resources, targeting isolation and discovery. Reverse-biological activity-guided isolation using an *in-silico* approach has promising potential to save cost and time invested in drug discovery. To achieve this, isolated known compounds are docked to potential target and their binding affinity compared with standards. This helps to understand whether the known or yet-to-be-identified compounds are responsible for the desired activity. Asteraceae family is reputed for the abundance of sesquiterpene lactones (STLs), and several of these lactones are known trypanocidal agents.

In this study, sesquiterpene lactones and other secondary metabolites (1-16) previously isolated from *T. diversifolia* and *V. glaberrima* (which extracts showed high *in-vivo* activities) were docked on ODC of *T. b. brucei* to ascertain their involvement in the anti- *T. b. brucei* activity and further provided a rationale for continual search for trypanocidal secondary metabolites from these plants [15-18]. Therefore, the study evaluated the *in-vivo* antitrypanosomal activity of the extracts of the five selected plants in mice-infected *T. b. brucei* and docked some selected isolated compounds from the most active extracts on *T. brucei* ODC.

2. Materials and Methods

2.1. Collection and authentication of plants.

The aerial part of *Tithonia diversifolia, Chromolaena odorata, Aspilia africana, Vernonia glaberrima,* and *Synedrella nodiflora* were collected in June 2020 from Nsukka, Nigeria, by a taxonomist, Mr. Felix Nwafor of the Department of Pharmacognosy and Environmental Medicine, University of Nigeria Nsukka. The voucher specimens (numbers UN/2020.Atd, UN/2020.Aco, UN/2020.Aaa, UN/2020.Avg, and UN/2020.Asn, respectively) of the plants were kept at the herbarium of the Department The plants’ pharmacognostic data were further confirmed at http://www.theplantlist.org at the time of the study.

2.2. Extraction of the plant material.

The collected aerial parts of the plants were dried for 2 weeks under shade and reduced into 1 mm coarse powder. The extraction protocol adopted was cold maceration. A 100 g each of the pulverized plant material was separately macerated in 500 mL of methanol (MeOH) for 48 h on a magnetic stirrer. After filtration, the extracts were concentrated *in vacuo* at 45ºC.

2.3. Induction of parasitemia in mice.

The mice were infected by administering 0.2 mL (containing 2.0 × 10⁵/mL trypanosomes) of the diluted blood (1 mL blood of mice infected with *T. brucei* species, collected through the media cantus of the eye, was diluted with 9 ml of normal saline)
intraperitoneally. The mice fed *ad libitum* with free access to clean water were monitored for 4 days until the level of parasitemia was significant enough (> $10^7$ trypanosomes/mL) but not too high to kill the mice, after which baseline parasitemia level and PCV were determined. The level of infection was ascertained in each rat tail blood examination for trypanosomes using a rapid matching counting method as previously described [19].

2.4. In-vivo antitrypanosomal assay.

The mice were divided into eighteen (n=5) groups: Groups 1A, IB, and 1C mice were infected and treated with 200, 400, and 600 mg/kg of *C. odorata*, respectively. Groups 2A, 2B, and 2C mice were infected and treated with 200, 400, and 600 mg/kg of *A. africana*, respectively. Mice in groups 3A, 3B, and 3C were infected and treated with 200, 400, and 600 mg/kg of *T. diversifolia*. Groups 4A, 4B, and 4C mice were infected and treated with 200, 400, and 600 mg/kg of *V. glaberrima*, respectively. All the mice in groups 5A, 5B, and 5C were infected and treated with 200, 400, and 600 mg/kg of *S. nodiflora*, respectively. Group 6 mice were infected but not treated (5ml/kg normal saline). Group 7 mice were infected and treated with 3.5 mg/kg of diminazene aceturate, while group 8 were uninfected and untreated. All the treatments started after infection and lasted for 12 days. The mice were monitored every other day during and after treatment for the level of parasitemia and PCV using the rapid matching and microhematocrit methods, respectively [19].

2.5. In-silico studies.

2.5.1. Preparation of *T. brucei* ODC structure.

The 3D structure of *T. brucei* ODC bearing its co-crystallized inhibitor, α-difluoromethylornithine (DFMO) (PDB ID: 2TOD, resolution 1.5747 Å) protein was obtained from https://www.rcsb.org/ [20]. The protein was cleaned and optimized by removing crystallographic waters and other bound heteroatoms before molecular docking in a Discovery Studio Visualizer (DSV) v17.2.0. Energy minimization of the protein was done with UCSF Chimera by the method of steepest descent of $10^2$ steps (size 0.02 Å) and the method of conjugate gradient comprising $10^1$ steps (size 0.02 Å). The native ligand and the target structures were used to validate the docking protocol and important interacting residues between the ligand and the receptors.

2.5.2. Preparation of the datasets.

The 2D structures of 1-16 (Figure 1) converted to 3D conformation from .mol to .pdb file. Energy minimization of the ligand was done using the MMFF94x force field and the conjugate gradients optimization algorithm by PyRx-Python prescription 0.8 for 200 steps [21].

2.5.3. Molecular docking.

Molecular docking was carried out using the docking algorithms protocols of AutoDock Tools 1.5.6 (ADT) by converting both the protein structure and ligands into (.pdbqt). Polar hydrogens were added to the protein receptors, non-polar hydrogens merged, and Gasteiger charges were then calculated, then assigning AutoDock4.2 (ADT4) atom types. Python scrips of the ADT and the customized python scripts were used to assign torsions to the ligands automatically. The search area was also determined using ADT4. The native ligand was first...
re-docked into the *T. brucei* ODC receptors to reproduce the original poses, after which 1-16 were docked into the *T. brucei* ODC receptors replicating the parameters used in the native ligand re-docking. For the docking experiment procedure, the protein and the ligands were loaded into ADT [25]. Rigid-receptor-flexible-ligand docking calculations were carried out by ADT in which the grid box with 0.375 Å points spacing was centered in the mass center surrounded by the amino acid residues at the receptor-binding site. Polar hydrogen atoms, Kollman charges, and solvation parameters were added using ADT. The Lamarckian Genetic Search Algorithm was used in this study to explore the space of active binding with different efficacy. AutoGrid module implemented in AutoDock was used to estimate potential grid maps for the interaction of ligand atom-types. In the docking computations, a total of 250 hybrid GA-LS runs, and at most 2.5 million energy evaluations and 0.27 million generations were performed. To group the clusters, a root-mean-square deviation (RMSD) tolerance was set at 2.0 Å. Other parameters were kept at default settings during the docking simulations. After that, DSV was used to visualize the interaction pattern in the protein-ligand complex.

![Figure 1](https://biointerfaceresearch.com/)

**Figure 1.** Test ligands from *T. diversifolia* (1-10) and *V. glaberrima* (11-16); diversifolin (1), diversifolin methylether (2), tagitinin D (3), woodhousin (4), tagitinin C (5), tagitinin F (6), tagitinin A (7), orizatin (8), tagitinin E (9), flavonol (10), homoeriodictyol (11), velutin (12), apigenin (13), lupeol (14), 5-methylcoumarin-4β-glucoside (15) and 4-hydroxy-5-methylcoumarin (16) [22-24].

2.6. Data analysis.

The collected data were analyzed using the Statistical Package for the Social Sciences (SPSS Inc. Chicago), v. 15.0 software. The parasitemia and the PCV were expressed as a mean ± standard deviation (SD) (n=5). One-way analysis of variance (ANOVA) was done to test for the significant difference between the means of samples and control at \( p < 0.05 \) by post-Hoc using 2-sided Dunnett’s test. In all cases, a \( p < 0.05 \) was considered to be significant.
3. Results and Discussion

3.1. Extraction of plant materials.

The cold maceration of *C. odorata*, *T. diversifolia*, *A. africana*, *V. glaberrima*, and *S. nodiflora* in MeOH (95 %v/v) yielded 8.04, 3.94, 3.18, 5.65, and 6.12 %w/w, respectively.

3.2. Effects of extracts on parasitemia.

The effect of the extracts on parasitemia was also evaluated and compared to the controls (Table 1). The plant extracts caused a dose-dependent reduction in parasitemia in the treated groups with no recrudescence within 17 days of induction and treatment. *T. diversifolia* extract (600 mg/kg) caused complete clearance of the parasites from the bloodstream within 14 days, while 200 and 400 mg/kg doses cleared them in 17 days compared with the 9-days effect recorded in the diminazene aceturate-treated groups. The 400 and 600 mg/kg of *V. glaberrima* also caused parasitemia on the 17th day of treatment.

| Groups | Mean parasitemia (ml)/Time (days) |
|--------|----------------------------------|
|        | 5      | 7      | 9      | 11     | 14     | 17     |
| 1A     | 8.43±0.68^a,b  | 8.40±0.00^a,b | 8.00±0.17^a,b | 7.70±0.17^a,b | 7.00±0.17^a,b | 7.00±0.17^a,b |
| 1B     | 8.00±0.62^a,b  | 7.92±0.50^a,b | 7.20±0.00^a,b | 7.65±0.45^a,b | 6.90±0.00^a,b | 3.45±0.45^a,b |
| 1C     | 8.30±0.11^a,b  | 8.22±0.14^a,b | 6.90±0.09^a,b | 6.82±0.27^a,b | 6.20±0.03^a,b | 2.90±0.28^a,b |
| 2A     | 8.50±0.17^a,b  | 8.40±0.00^a,b | 7.8±0.00^a,b  | 7.50±0.00^a,b | 6.90±0.00^a,b | 6.90±0.00^a,b |
| 2B     | 8.30±0.46^a,b  | 8.60±0.17^a,b | 8.10±0.30^a,b | 7.50±0.30^a,b | 7.00±0.17^a,b | 6.90±0.00^a,b |
| 2C     | 8.70±0.03^a,b  | 8.22±0.21^a,b | 8.00±0.25^a,b | 6.90±0.92^a,b | 6.81±0.24^a,b | 6.00±0.15^a,b |
| 3A     | 8.80±0.17^a,b  | 8.30±0.17^a,b | 7.60±0.17^a,b | 7.60±0.35^a,b | 2.30±3.98^a,b | 0.00±0.00^a,b |
| 3B     | 8.40±0.52^a,b  | 8.30±0.17^a,b | 8.00±0.17^a,b | 7.20±0.30^a,b | 2.30±3.98^a,b | 0.00±0.00^a,b |
| 3C     | 8.40±0.24^a,b  | 8.10±0.10^a,b | 7.25±0.90^a,b | 6.60±0.32^a,b | 0.00±0.00^a,b | 0.00±0.00^a,b |
| 4A     | 8.40±0.52^a,b  | 8.20±0.17^a,b | 8.00±0.17^a,b | 7.80±0.30^a,b | 7.30±0.17^a,b | 6.90±0.00^a,b |
| 4B     | 8.40±0.00^a,b  | 8.20±0.17^a,b | 7.70±0.17^a,b | 7.10±0.17^a,b | 7.00±0.17^a,b | 0.00±0.00^a,b |
| 4C     | 8.46±0.20^a,b  | 8.02±0.31^a,b | 6.90±0.18^a,b | 6.22±0.82^a,b | 4.29±0.12^a,b | 0.00±0.00^a,b |
| 5A     | 8.00±0.35^a,b  | 7.90±0.17^a,b | 7.40±0.17^a,b | 7.60±0.17^a,b | 6.90±0.00^a,b | 6.90±0.00^a,b |
| 5B     | 8.50±0.17^a,b  | 8.10±0.30^a,b | 7.60±0.35^a,b | 8.90±0.17^a,b | 6.90±0.00^a,b | 6.90±0.00^a,b |
| 5C     | 8.80±0.63^a,b  | 8.03±0.51^a,b | 7.20±0.17^a,b | 6.70±0.90^a,b | 5.8±0.27^a,b | 5.10±0.72^a,b |
| 6      | 8.40±0.30 | 9.00±0.00 | 8.90±0.17 | 8.90±0.17 | 9.00±0.00 | 8.90±0.17 |
| 7      | 8.80±0.17 | 7.00±0.17 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| 8      | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |

Data expressed as mean ± SD (n = 5); ^a,b^p < 0.05, ^a,b^p < 0.05 are significant compared to control groups 7 and 8 respectively.

3.3. Effects of extracts on PCV.

The effects of the studied plant extracts on PCV are shown in Figure 2. There was no difference in the effects of 400 and 600 mg/kg of extracts in all the treatments. The 400 mg/kg dose of *S. nodiflora* caused a sharp decrease in PCV on the 9th day, which was lowest on day 11 before the sharp rise after day 11.

3.4. In-silico effects of constituents of *V. glaberrima* and *T. diversifolia* on *T. brucei* ODC.

The screening of 1-16 on *T. brucei* ODC employed AutoDock4.2 software with the forcefield-based scoring function, and its docking protocol was validated based on the RMSD method. A grid box points 4 x 4 x 3 with 1.5747Å spacing centered on the mass center of 4 x 4 x 3, which reproduced the experimental pose of the co-crystallized inhibitor, was used to dock the 16 compounds. The binding free energies (E_b) of the native and test ligands with the best binding poses are shown in Table 2 and Figure 3.
The results of docking validation (Figure 3A) show major interactions between *T. brucei* ODC and DFMO, the native inhibitor. The major interaction of the native ligand was with Tyr323 and Cys361 of ODC. The analysis of the binding pose of the hits from each of the most active plants showed some biochemically interesting binding modes. Woodhousin (4) interacted with Ser 200 and showed exposures to many amino acids of *T. brucei* ODC (Figure 3B).

**Table 2.** Docked ligands parameters with good binding affinity/interaction.

| Ligands | S     | RMSD_refine | E_conf | E_place | E_score1 | E_refine | E_score of DFMO = -10.0251 Kcal/mol |
|---------|-------|-------------|--------|---------|----------|----------|-------------------------------------|
| 1       | -9.9184 | 2.37389     | 22.8339 | -56.085 | -10.619  | -9.91848 |
| 2       | -12.618 | 1.33126     | 41.2337 | -79.992 | -10.992  | -12.6184 |
| 3       | -10.874 | 3.09453     | 45.3454 | -46.493 | -10.645  | -10.8744 |
| 4       | -13.612 | 1.91867     | -28.308 | -67.886 | -11.233  | -13.6120 |
| 5       | -15.694 | 3.27171     | 112.914 | -49.746 | -10.158  | -15.6943 |
| 6       | -13.582 | 3.05106     | 24.5182 | -52.508 | -10.373  | -13.5821 |
| 7       | -12.139 | 3.15596     | 23.6242 | -48.001 | -10.811  | -12.1399 |
| 8       | -10.874 | 3.09453     | 45.3454 | -46.493 | -10.646  | -10.8742 |
| 9       | -18.607 | 3.41757     | 158.525 | -65.964 | -9.4572  | -18.6072 |
| 10      | -13.966 | 2.30360     | 22.8460 | -37.174 | -8.5851  | -13.9668 |
| 11      | -11.546 | 1.22987     | 24.6971 | -63.860 | -8.6975  | -11.5467 |
| 12      | -10.647 | 2.83478     | 10.2009 | -55.876 | -8.6229  | -10.6476 |
| 13      | -10.247 | 3.08585     | 20.8663 | -53.864 | -10.093  | -10.2473 |
| 14      | -10.113 | 3.33576     | 25.3683 | -62.088 | -9.5603  | -10.1135 |
| 15      | -10.083 | 1.584087    | 20.0989 | -56.559 | -8.5947  | -10.0838 |

**Figure 2.** Effects of extracts on PCV. Only the data for 200 and 400 mg/kg were plotted.

**Figure 3.** The original DFMO (A) and woodhousin (B) interactions with important residues in *T. brucei* ODC.
3.5. Discussion.

Several plants used in folk medicine have been validated to possess such activities in the past, and their active constituents have also been isolated from such plants [6,19]. This study identified some untapped plants used in ethnomedicine to treat some parasitic infections and validated their anti-*T. brucei brucei* property by *in-vivo* mice model, thereby complementing other pharmacological activities of these plants [7-18]. The study further identified several isolated compounds from the two most active extracts and docked them on *T. brucei* ODC to understand their mechanisms of antitrypanosomal activity.

This study observed a dose-dependent clearance of the parasites from the blood of infected animals within 17 days of treatment. The results showed that 200, 400, and 600 mg/kg doses of *C. odorata* caused 12.5, 56.7, and 63.5 % clearance of *T. brucei* within 17 days. Similarly, *V. glaberrima* and *T. diversifolia* caused total clearance of the parasites in 17 days compared to the corresponding effect of diminazene aceturate on the 9th day. Apart from the attempted relapse of the parasites in mice treated with *S. nodiflora* (200 and 400 mg/kg) on days 9 to 11, other treatments did not show any potential relapse. A comparison of the 100 % a parasitemia caused by *V. glaberrima* (17th day) and *T. diversifolia* (14th day) with diminazene aceturate control (9th day) showed that the extracts possess slow onset of trypanocidal activity, which is an advantageous characteristic and similar to conventional diamidines [17].

To further confirm the trypanocidal activities, the PCV was monitored for the same period. It is known that a high level of trypanosomes infection leads to hemolysis of red blood cells, which reduces the PCV significantly [26]. All the treatments resulted in the restoration of the PCV to their respective baselines, though at different rates compared to the uninfected and untreated groups. However, *S. nodiflora* extracts (400 mg/kg) caused a sharp drop in the PCV on the 11th day, similar to the clearance rate of the parasites from the bloodstream, suggesting an attempted recrudescence of the infection. Similarly, both *V. glaberrima* and *T. diversifolia* caused complete restoration of the PCV to their respective baselines before the treatments. This further confirmed the higher activities of the extracts recorded in the clearance of parasites from the bloodstream of infected mice.

The higher *in-vivo* anti-*T. brucei* activities of *V. glaberrima* and *T. diversifolia* in this study excited our interest in probing for the bioactive constituents of these widely distributed plants. To explore this, some important molecules previously isolated from them were docked on *T. brucei* ODC (a known and validated drug target) to understand their roles in this activity. The best-docked conformations with the best binding poses from *T. diversifolia* included: β-methyldiversifolin (1), -10.61 Kcal/mol; orizatin (8), -10.646 Kcal/mol; woodhousin (4), -11.233 Kcal/mol, Tagitinin A (7), -10.811 Kcal/mol and diversifolin methylether (2), -10.992 Kcal/mol. Compared with DFMO (E-score -10.0251 Kcal/mol), woodhousin of the *T. diversifolia* showed more effective interaction with *T. brucei* ODC. Interestingly, apigenin (13) from *V. glaberrima* showed a slightly higher binding (E-score -10.093 Kcal/mol) to *T. brucei* ODC than the native ligand but lower than those from *T. diversifolia*. Of the 16 compounds virtually screened, all the sesquiterpene lactones (1-9) showed strong binding to and inhibition of *T. brucei*’s ODC compared with E-score -10.0251 Kcal/mol of DFMO. From the docked poses, woodhousin interacted maximally with the serine amino acid (Ser200) sequence of the target by forming a hydrogen bond at the carbonyl functional group (with interatomic distance 2.18 Å). The *in-silico* data from the study was consistent with the *in vivo* assay data, thus
eliciting further search for these phytochemicals and other unknown bioactive sesquiterpene lactones from *T. diversifolia* leaves.

Sesquiterpene lactones are an important class of sesquiterpenoids in plants of the family Asteraceae, Umbeliferae, and Magnoliaceae that possess a variety of biological activity due to their characteristic α-methylene-γ-lactone motif [27]. It is imperative to note that this study did not characterize the trypanocidal phytochemical constituents of *T. diversifolia* and *V. glaberrima* or other lesser active extracts. However, antitrypanosomal activities of STLs have been implicated extensively [28,29]. To further confirm the connection between the high *in-vivo* anti-*T. brucei brucei* activities observed in the extract of *T. diversifolia* and the strong binding energies (stronger than the native ligand, DFMO) of the STLs from *T. diversifolia*, we are currently isolating and testing these phytochemicals to provide further phytochemical and biological evidence in support of the use of these plants in folk medicine.

4. Conclusions

The *in-vivo* study has shown that *T. diversifolia* and *V. glaberrima* are a potential source of strong anti-*T. brucei* agents. The *in-silico* data supported the *in-vivo* findings and further showed that STLs from *T. diversifolia* could serve as a source of lead compounds for the management of AAT.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Shereni, W.; Neves, L.; Argilés, R.; Nyakupinda, L.; Cecchi, G. An atlas of tsetse and animal *African trypanosomiasis* in Zimbabwe. *Parasites and Vectors* 2021, 14, 1-10, https://doi.org/10.1186/s13071-020-04555-8.
2. Richards, S.; Morrison, L.J.; Torr, S.J.; Barrett, M.P.; Manangwa, O.; Mramba, F.; Auty, H. Pharma to farmer: Field challenges of optimizing trypanocide use in African animal trypanosomiasis. *Trends Parasitol* 2021, 37, 831-843, https://doi.org/10.1016/j.pt.2021.04.007.
3. Giordani, F.; Paape, D.; Vincent, I.M.; Pountain, A.W.; Fernández-Cortés, F.; Rico, E.; Zhang, N.; Morrison, L.J.; Freund, Y.; Witty, M.J.; Peter, R. Veterinary trypanocidal benzoxaboroles are peptidase-activated produgs. *PLoS Pathogens* 2020, 16, https://doi.org/10.1371/journal.ppat.1008932.
4. Crump, R.E.; Huang, C.I.; Knock, E.S.; Spencer, S.E.; Brown, P.E.; Mwamba Miaka, E.; Shampa, C.; Keeling, M.J.; Rock, K.S. Quantifying epidemiological drivers of gambiense human *African trypanosomiasis* across the Democratic Republic of Congo. *PLoS Comput Biol* 2021, 17, https://doi.org/10.1371/journal.pcbi.1008532.
5. Moujir, L.; Callies, O.; Sousa, P.; Sharopov, F.; Seca, A.M. Applications of sesquiterpene lactones: A review of some potential success cases. *Appl Sci* 2020, 10, https://doi.org/10.3390/app10093001.
6. Nnadi, C.O.; Okorie, H.N.; Nwodo, N.J. Evaluation of in vitro antiprotozoal and cytotoxic activities of selected medicinal plants used in Nigerian folk medicine. *Trop J Nat Prod Res* **2021**, *5*, 609-612, https://doi.org/10.26538/jnpnr/v5i4.2.

7. Dongmo, A.N.; Nguefack, J.; Dongmo, J.B.L.; Foulelefack, F.R.; Azah, R.U.; Nkengfack, E.A.; Stefani, E. Chemical characterization of an aqueous extract and the essential oil of *Tithonia diversifolia* and their biocontrol activity against seed-borne pathogens of rice. *J Plant Dis Protect* **2021**, *128*, 703-713, https://doi.org/10.1007/s41348-021-00439-w.

8. Gitahi, S.M.; Piero, M.N.; Mburu, D.N.; Machocho, A.K. Repellent Effects of Selected Organic Leaf Extracts of *Tithonia diversifolia* (Hemsl.) A. Gray and *Veronica lasiopus* (O. Hoffman) against *Sitophilus* zeamais Motschulsky (Coleoptera: Curculionidae). *The Sci World J* **2021**, *2021*, https://doi.org/10.1155/2021/2718629.

9. Dougnon, G.; Ito, M. Essential Oil from the Leaves of *Chromolaena odorata*, and Sesquiterpene Caryophyllene Oxide Induce Sedative Activity in Mice. *Pharmaceuticals* **2021**, *14*, https://doi.org/10.3390/phi14070651.

10. Ifeanacho, M.O.; Ikewuchi, J.C.; Ikewuchi, C.C.; Nweke, P.C.; Okere, R.; Nwate, T.L. Prevention of doxorubicin-induced dyslipidaemia, plasma oxidative stress and electrolytes imbalance in Wistar rats by aqueous leaf-extracts of *Chromolaena odorata* and *Tridax procumbens*. *Sci Afr* **2021**, *11*, https://doi.org/10.1016/j.sciaf.2020.e00636.

11. Gangas, P.; Aliyu, A.B.; Oyewale, A.O. GC-MS Analysis and Antibacterial Effects of *Vernonia glaberrima* n-Hexane Extracts alone and in Combination with Standard Antibiotics. *J Chem Soc Nig* **2021**, *46*, https://doi.org/10.46602/jcsn.v46i2.593.

12. Niyonzigiye, I.; Ngabirel, D.; Nkurunziza, D.; Patil, M.P.; Singh, A.A.; Ngezahayo, J.; Kim, G.D. *Aspilia africana* CD Adams inhibits the production of lipopolysaccharide-induced inflammatory mediators in murine macrophage RAW264.7 cells by suppressing the NF-kB and PI3K/Akt pathways. *South Afr J Bot* **2021**, *141*, 83-89, https://doi.org/10.1016/j.sajb.2021.04.028.

13. Chaniad, P.; Techarang, T.; Phuawajaranpong, A.; Na-Ek, P.; Viriyavejakul, P.; Punsawad, C. In Vivo Antimalarial Activity and Toxicity Study of Extracts of *Tugetes erecta* L. and *Syndetrella nodiflora* (L.) Gaertn. from the Asteraceae Family. *Evidence-Based Compl Alt Med* **2021**, *2021*, https://doi.org/10.1155/2021/1270902.

14. Astuti R.J.; Prasty, M.E.; Batubara, I.; Budiarti, E.; Imliyawati, A. Antiaging and Antioxidant Bioactivities of Asteraceae Plant Fractions on the Cellular Functions of the yeast *Schizosaccharomyces pombe*. *Adv Pharmacol Pharm Sci* **2021**, *2021*, https://doi.org/10.1155/2021/2119634.

15. Pantoja-Pulido, K.D.; Rodriguez, J.; Isaza-Martinez, J.H.; Gutiérrez-Cabrera, M.; Colmenares-Dulcey, A.J.; Montoya-Lerma, J. Insecticidal and cholinesterase activity of dichloromethane extracts of *Tithonia diversifolia* on Atta cephalotes worker ants (Formicidae: Myrmicinae). *Insects* **2020**, *11*, https://doi.org/10.3390/insects11030180.

16. Chunudom, L.; Thongsom, M.; Karim, N.; Rahman, M.A.; Rana, M.N.; Tangpong, J. *Tithonia diversifolia* aqueous fraction plays a protective role against alloxan-induced diabetic mice via modulating GLUT2 expression. *South Afr J Bot* **2020**, *133*, 118-123, https://doi.org/10.1016/j.sajb.2020.07.007.

17. Amoateng, P.; Tagoe, T.A.; Karikari, T.K.; Kukaia, K.K.; Osei-Safo, D.; Woode, E.; Freenguelli, B.G.; Kombian, S.B. *Syndetrella nodiflora* extract depresses excitatory synaptic transmission and chemically-induced in-vitro seizures in the rat hippocampus. *Frontiers Pharmacol* **2021**, *12*, https://doi.org/10.3389/fphar.2021.610025.

18. Wouamba, S.C.; Hippi, G.M.; Lenta, B.N.; Sewald, N.; Kouam, S.F. Vernoguinamide: A new ceramide and other compounds from the root of *Vernonia guineensis* A. Gray and *Cromolaena odorata* against *Schizosaccharomyces pombe*. *Adv Pharmacol Pharm Sci* **2020**, *88*, https://doi.org/10.1016/j.sajb.2019.103988.

19. Nnadi, C.O.; Ozioko, L.U.; Eneje, G.C.; Onah, C.M.; Obong, W.O. *In vivo* antitrypanosomal effect and *in silico* prediction of chronic toxicity of N-methylolophyllamine in rats. *Trop J Pharm Res* **2020**, *19*, 2369-2375.

20. Grishin, N.V.; Osterman, A.L.; Brooks, H.B.; Philips, M.A.; Goldsmith, E.J. X-ray structure of ornithine decarboxylase from *Trypanosoma brucei*: The native structure and the structure in complex with alpha-difluoromethylornithine. *Biochem 1999*, *38*, 15174-15184, https://doi.org/10.1021/bi9915115.

21. Almrud, J.J.; Oliveira, M.A.; Kern, A.D.; Grishin, N.V.; Phillips, M.A.; Hackert, M.L. Crystal structure of ornithine decarboxylase at 2.1 Å resolution: structural insights to antizyme binding. *J Molec Biol* **2000**, *295*, 7-16, https://doi.org/10.1006/jmbi.1999.3331.

22. Alhassan, A.M.; Ahmed, Q.U.; Latip, J.; Shah, S.A.; Sarian, M.N.; Wahab, R.A.; Taher, M.; Abdullahi, M.I.; Khatib, A. Phytoconstituents from *Vernonia glaberrima* Welw. Ex O. Hoffm. leaves and their cytotoxic activities on a panel of human cancer cell lines. *South Afr J Bot* **2018**, *116*, 16-24, https://doi.org/10.1016/j.sajb.2018.02.391.

23. Ngarivhume, T.; Noreljaleel, A.; Bonnet, S.L.; Wilhelm, A. Isolation and antimalarial activity of a new flavonol from *Tithonia diversifolia* leaf extract. *Chem 2021*, *3*, 854-860, https://doi.org/10.3390/chemistry3030062.
24. Baruah, N.C.; Sharma, R.P.; Madhusudanan, K.P.; Thyagarajan, G.; Herz, W.; Murari, R. Sesquiterpene lactones of *Tithonia diversifolia*. Stereochemistry of the tagitinins and related compounds. *The J Org Chem* **1979**, *44*, 1831-1835, https://doi.org/10.1021/jo01325a018.

25. Toyang NJ and Verpoorte R. A review of the medicinal potentials of plants of the genus Vernonia (Asteraceae). *J. Ethnopharmacol.* **2013**, *146*, 681-723, https://doi.org/10.1016/j.jep.2013.01.040.

26. Ikede, B.O.; Lule, M.; Terry, R.J. Anaemia in trypanosomiasis: Mechanisms of erythrocyte destruction in mice infected with *Trypanosoma congolense* or *T. brucei*. *Acta Tropica* **1977**, *34*, 53-60.

27. Muschietti, L.V.; Ulloa, J.L. Natural sesquiterpene lactones as potential trypanocidal therapeutic agents: A review. *Nat Prod Comm* **2016**, *11*, 1569-1578.

28. Elso, O.G.; Bivona, A.E.; Sanchez Alberti, A.; Cemy, N.; Fabian, L.; Morales, C.; Catalan, C.A.; Malchiodi, E.L.; Cazorla, S.I.; Sulsen, V.P. Trypanocidal activity of four sesquiterpene lactones isolated from Asteraceae species. *Molecules* **2020**, *25*, https://doi.org/10.3390/molecules25092014.

29. Sosa, A.; Salamanca Capusiri, E.; Amaya, S.; Bardon, A.; Gimenez-Turba, A.; Vera, N.; Borkosky, S. Trypanocidal activity of South American Vernoniaeae (Asteraceae) extracts and its sesquiterpene lactones. *Nat Prod Res* **2020**, *35*, 1-5, https://doi.org/10.1080/14786419.2020.1739682.