



## Molecular Docking Analysis and Anti-trypanosomal Properties of *Cymbopogon citratus* Ethanol Leaves Extracts

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### ABSTRACT

African trypanosomiasis, a neglected tropical disease transmitted by tsetse flies and caused by *Trypanosoma brucei brucei*, poses significant health and economic burdens across sub-Saharan Africa. Conventional treatment methods face challenges, including drug resistance and high costs, underscoring the need for alternative therapies. This study investigates the anti-trypanosomal properties of *Cymbopogon citratus* (lemongrass) leaf extracts at concentrations ranging from 100 to 400 mg/kg, evaluating their effects on parasitaemia levels *in vivo* and through molecular docking studies. Isometamidium chloride and normal saline served as positive and negative controls, respectively. Phytochemical analysis identified the presence of alkaloids, phenolics, terpenes, anthraquinones, steroids, cardiac glycosides, and tannins in both ethanolic and aqueous extracts of *C. citratus*. Flavonoids and carbohydrates were detected in the aqueous extract. *In vivo* studies showed both extracts significantly reduced parasitaemia in infected mice, with the 200 mg/kg aqueous extract producing the most pronounced suppression by day 4 ( $p = 0.0105$ ) and outperforming the ethanolic extract ( $p = 0.0347$ ). The packed cell volume declined over time in all tests, except for the aqueous extract in the suppressive test, which showed a slight increase. No mortality was recorded in acute toxicity tests, indicating both extracts were non-toxic. Furthermore, molecular docking evaluations revealed that compounds like geranyl acetate from *C. citratus* interact with key residues of the *T. brucei brucei* L-threonine-3-dehydrogenase enzyme, potentially inhibiting its activity. These findings suggest that *C. citratus* holds promise as a source of novel anti-trypanosomal agents and may contribute to the development of alternative, plant-based therapies for African trypanosomiasis.

**Keywords:** *Trypanosoma brucei brucei*, *Cymbopogon citratus*, Molecular Docking, geranyl acetate, L-threonine-3-dehydrogenase

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### Introduction

African trypanosomiasis, a neglected tropical disease caused by trypanosomes and transmitted by tsetse flies (*Glossina* species), remains a significant health and economic challenge across sub-Saharan Africa, according to the World Health Organization.<sup>1</sup> The disease affects both humans and animals, with severe consequences for livestock production and agricultural productivity, resulting in losses of approximately \$5 billion annually.<sup>2</sup> Although chemotherapy and other control measures are being implemented,<sup>3</sup> the persistent issues of drug resistance, toxicity, and expensive treatment costs underscore the pressing need for alternative solutions that are cost-effective, safe, and therapeutically effective.<sup>4</sup>

In particular, the absence of a vaccine and the increasing prevalence of resistance to conventional drugs, such as diminazene aceturate and suramin, underscore the necessity for exploring novel anti-trypanosomal agents.

Recent research has focused on natural products, which have demonstrated promising anti-parasitic properties.<sup>6,7</sup> *Cymbopogon citratus*, a plant with a long history of use in traditional medicine, has emerged as a potential source of bioactive compounds with diverse therapeutic applications, including antimalarial, antifungal, antibacterial, and anti-inflammatory properties.<sup>8,9,10</sup> However, its potential as an anti-trypanosomal agent, particularly against *Trypanosoma brucei brucei*, remains underexplored.

This study investigates the anti-trypanosomal properties of *C. citratus* leaf extracts, utilizing *in-vivo* models to evaluate its efficacy and safety. Additionally, molecular docking studies were conducted to identify potential binding interactions between the plant's bioactive compounds and key drug targets in *T. brucei brucei*, providing insights into their mechanisms of action through a combination of experimental and computational approaches.

### Materials and Methods

#### Collection and Processing of Plant Materials

*Cymbopogon citratus* was collected from Dadin Kowa (Latitude: 8.75309° N, Longitude: 9.87639° E), Jos South Local Government Area, Plateau State, Nigeria, and identified by a taxonomist at the

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Department of Plant Science and Biotechnology, University of Jos with a voucher number; UJ/PCG/HSP/93 G 05. The leaves were washed, air-dried in the shade for 7 days, and powdered. The powder sample (100 g) was soaked in 70% ethanol for 24 hours, shaken for 3 hours, and filtered through Chinese cloth. The filtrate was evaporated using a rotary evaporator, and the resulting dry sample was then tested for its phytochemical constituents. The same method was used for water extracts, which were stored in airtight containers.<sup>11</sup>

#### Phytochemical Screening

Qualitative phytochemical analysis of ethanolic and aqueous extracts was performed using standard methods as described.<sup>10</sup> The plant fractions were examined to detect the presence of alkaloids, saponins, tannins, flavonoids, carbohydrates, steroids, anthraquinones, cardiac glycosides, phenols, and terpenoids.

#### Animal Use and Ethical Consideration

Laboratory rodents (Swiss albino Mice—*Mus musculus*) of both sexes and known weight were used during the study period. The animals were obtained from the animal house at the University of Jos and fed standard livestock feed and clean water. This study was approved by the University of Jos Institutional Animal Care and Use Committee (IACUC) in collaboration with the Office of Laboratory Animal Welfare (OLAW), with reference number F17-00379.

#### Acute Toxicity Test

Nine (9) mice were randomly divided into three groups of three animals for the toxicity test. Each group was administered 1,300 mg/kg, 2,500 mg/kg, or 5,000 mg/kg of crude leaf extract.<sup>12</sup>

#### Parasite Inoculation

*T. brucei brucei* was obtained from the Nigerian Institute for Trypanosomiasis Research, Vom, Plateau state (NITR). The mice were randomly inoculated with 0.2 mL of diluted blood containing  $1.2 \times 10^7$  parasitized red blood cells to study the effect of the crude leaf extract. The animals were divided into three groups, each consisting of 25 animals for the tests (Suppressive, Curative, and Prophylactic).<sup>4</sup> In each of the test groups, animals were divided into five groups of five animals each and treated once daily for four consecutive days.

#### Suppressive Test

This study adopted a previously described method.<sup>4</sup> Twenty-five mice were divided into five groups of five, each inoculated with *T. brucei brucei*. Three groups received 100 mg/kg, 200 mg/kg, and 400 mg/kg of crude extracts. In contrast, one group received 1.2 mg/kg of isometamidium chloride as a positive control, and the other received normal saline as a negative control. Treatment continued daily until day 4. Blood was collected on day 3, and thin smear slides were prepared using Giemsa stain. The percentage parasitaemia was calculated by counting parasitized cells in 4 random fields under oil immersion (equation 1).

$$\text{Percentage parasitaemia (\%)} = \frac{\text{Number of parasitized red blood cells}}{\text{Total number of red blood cells}} \times 100 \dots\dots\dots 1$$

The average percentage suppression for the dose of the extract administered was calculated in comparison to the control using the following formula (equation 2).

$$\% \text{ Suppression} = \frac{\% \text{ Parasitaemia in Control} - \% \text{ Parasitaemia in test group}}{\% \text{ Parasitaemia in Control}} \times 100 \dots\dots\dots 2$$

#### Curative Test

The methodology employed in this study was adapted from Dawet *et al.*<sup>13</sup> Twenty-five mice were infected with *T. brucei brucei*, and 72 hours post-infection, they were divided into five groups of five. Three groups received 100 mg/kg, 200 mg/kg, and 400 mg/kg of crude

extracts. In contrast, one group received 1.2 mg/kg of Isometamidium chloride as a positive control, and the other received normal saline as a negative control. Mice were treated orally once daily for four consecutive days, and parasitaemia levels were determined on day 10 (equation 3).

$$\% \text{ Inhibition} = \frac{\text{Mean \% Parasitaemia of untreated group} - \text{Mean \% Parasitaemia in test group}}{\text{Mean \% Parasitaemia of untreated group}} \times 100 \dots\dots\dots 3$$

#### Prophylactic Test

The methodology for the prophylactic test was also adapted from Dawet *et al.*<sup>13</sup> The animals were divided into five groups of five. Three groups received 100 mg/kg, 200 mg/kg, and 400 mg/kg of crude extracts, respectively. One group received 1.2 mg/kg of ethanol as a positive control, and the remaining group received normal saline as a negative control. After four days of treatment, the mice were inoculated with *T. brucei brucei*. Seventy-two hours post-inoculation, blood was collected from the tail, and thin and thick film smears were prepared and examined microscopically for parasitemia. The percentage inhibition was calculated using equation 4.

$$\% \text{ Inhibition} = \frac{\text{Mean \% Parasitaemia of untreated group} - \text{Mean \% Parasitaemia in test group}}{\text{Mean \% Parasitaemia of untreated group}} \times 100 \dots\dots\dots 4$$

#### Haematological Parameters

The peripheral blood obtained from the tail vein of each mouse was used for determining the packed cell volume (PCV), as previously described.<sup>4</sup> PCV measured 4 days pre- and post-inoculation. The packed cell volume was estimated by filling capillaries to about three-quarters of the tubes with blood samples. These tubes were sealed at one end with a crista seal and placed in a microhaematocrit centrifuge with the sealed ends outermost.<sup>14</sup> Centrifuging was at a predetermined speed of 5 minutes, using a PCV reader; the estimated value for each mouse was taken and recorded.

#### In silico Modelling of Molecular Interactions

The inhibitory mechanisms and biological interactions of the chemical compounds in *C. citratus* against *T. brucei brucei* L-threonine-3-dehydrogenase were assessed through *in silico* molecular modelling.

#### Ligand Retriever

The chemical compositions of *C. citratus* were identified from the literature<sup>15</sup> and retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>) in MOL SDF (.sdf) format. The compounds were Juniper camphor (CID 521214), Geranyl acetate (CID 1549026), 1,2-Benzendicarboxylic acid (CID 1017), Z-Citral (CID 638011), Trans-geraniol (CID 637566), G-methyl-5-hepten-2-One (CID 9862). Standard inhibitor NAD (CID 5892) and Isometamidium Chloride (CID 72452) were also retrieved.

#### Ligand Preparation

The ligands with PubChem CID 72452, 9862, 1017, 637566, 638011, 1549026, 5892, and 2519 in mol SDF format were converted to a PDBQT file using the PyRx tool to generate atomic coordinates, and energy was minimized by optimization using the optimization algorithm at the force field set at off on PyRx.

#### Target Identification and Retrieval

The target protein, L-Threonine-3-Dehydrogenase (PDB ID: 5K4W), was retrieved from the Protein Data Bank (PDB) at <http://www.rcsb.org/pdb/home/home.do>.

### Target Preparation

The Pymol tool was used to remove the bound molecules from the proteins, as well as non-essential water molecules and all heteroatoms. The crystallized ligands were extracted (not removed) from the active sites of these proteins to reveal the grid coordinate around the binding pocket when viewed in PyMOL and Discovery Studio 2017 R2.<sup>16</sup>

### Molecular Docking

After the retrieval and preparation of receptors and ligands, molecular docking procedures were performed using PyRx and AutoDock Vina, based on the scoring function options. After energy minimizations, the grid box coordinates that define the binding sites for the proteins are: PDB ID: 5k4w, center x = 46.2998, center y = -25.247, center z = -35.4519, size x, y, and z = 25 Å. The standard (Isometamidium Chloride) was docked on the same binding sites as *C. citratus* compounds for a target protein with the exact grid box coordinates, and their respective interactions were compared within similar active sites.<sup>16</sup>

### Statistical Analysis

The data was cleaned and coded in MS Excel, then imported into Stata Version 14.0 for statistical analysis. A three-way ANOVA was conducted to assess the interaction between solvent type, concentration, and exposure period on the mean weight, PCV, and parasitemia levels of the test rats. Bonferroni pairwise tests were used to compare significant mean differences. The p-value of <0.05 was considered significant.

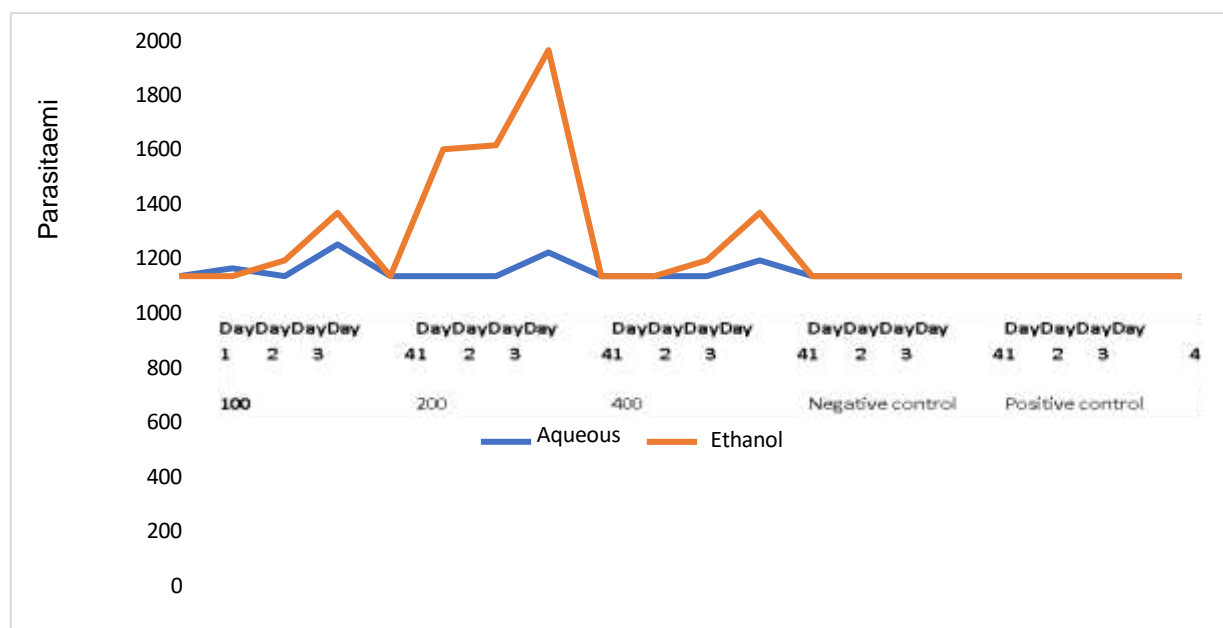
## Results and Discussion

Phytochemical analysis identified the presence of alkaloids, phenolics, terpenes, anthraquinones, steroids, cardiac glycosides, and tannins in both ethanolic and aqueous extracts of *C. citratus*. Flavonoids and carbohydrates were exclusively detected in the aqueous extract.

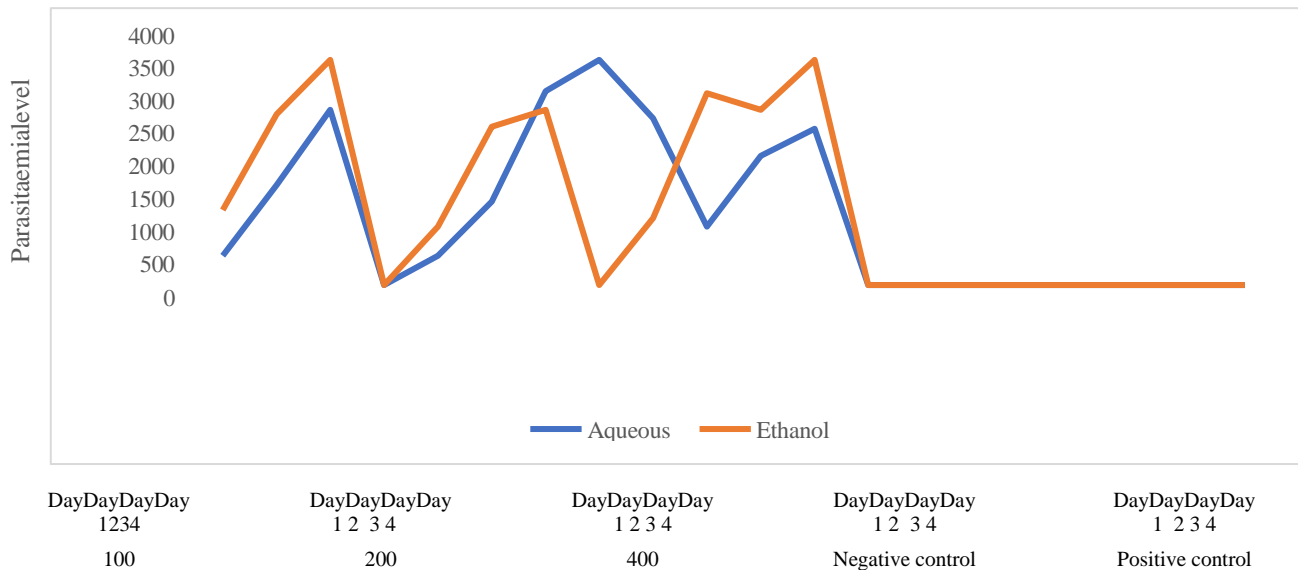
Plant leaves are valuable for health and nutrition due to their nutrient composition and bioactive metabolites, which exhibit anti-inflammatory, antibacterial, antioxidant, hypoglycemic, and immunomodulatory effects.<sup>17, 18</sup> This finding is consistent with that of Urama *et al.*,<sup>1</sup> who reported that all parts (leaves, stalk, and root) of *C. citratus* contain flavonoids, glycosides, alkaloids, saponins, phenols,

and tannins. Alkaloids have been shown to treat conditions such as diabetes mellitus, fever, and headaches.<sup>19</sup> Beyond their physiological activities, these compounds are also known for their therapeutic properties.<sup>20</sup>

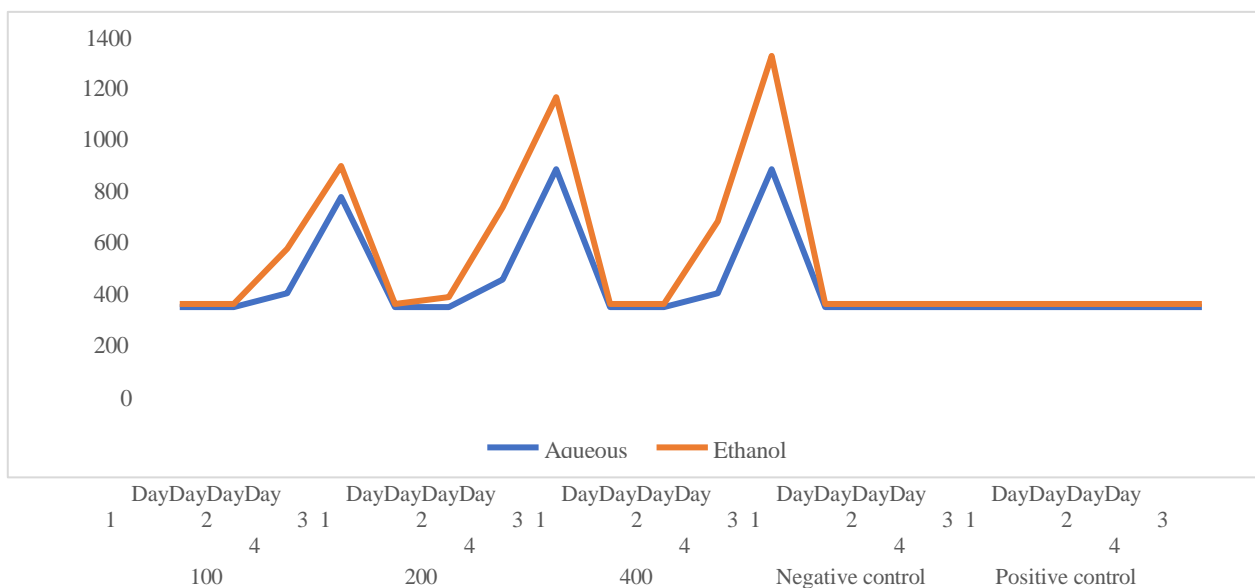
In the anti-parasitic assays, the extracts suppressed parasitaemia in treated mice, with prolonged exposure resulting in higher suppression, as presented in Figure 1. The 200 mg/kg concentration had the highest parasitaemia at 4 days post-exposure for both aqueous and ethanol extracts, with negative controls showing no parasitaemia. ANOVA revealed a significant interaction between solvent type and concentration on parasitaemia levels ( $F(4, 46) = 5.38, P = 0.0012$ ). Bonferroni pairwise tests showed higher parasitaemia with the ethanolic extract at 200 mg/kg ( $p < 0.05$ ). This finding is consistent with Yandev *et al.*,<sup>21</sup> who reported that the methanolic extract of *C. citratus* (lemon grass) completely inactivated *T. brucei brucei*. Ogbole *et al.* reported that the methanol fractions of the stem bark extract of *Acacia nilotica* significantly reduced parasitemia in *T. congolense*-infected albino mice.<sup>22</sup> This agrees with Amaechi *et al.*,<sup>23</sup> who reported a 97.75% reduction in parasitemia at a dose of 6.25 mg/kg of isometamidium chloride. Regarding the curative effect, parasitaemia increased with exposure time but decreased with higher concentrations, as presented in Figure 2. ANOVA showed that the aqueous extract had a significantly lower effect than the ethanolic extract ( $F(1, 30) = 4.90, P = 0.0347$ ), and parasitaemia decreased with both exposure time ( $F(3, 30) = 4.49, P = 0.0105$ ) and concentration ( $F(5, 30) = 12.06, P = 0.0000$ ). Isometamidium chloride, the standard drug, completely cleared the parasites.<sup>23</sup> The extracts demonstrated a dose-dependent effect, where lower doses resulted in a less significant reduction in parasite density. In comparison, higher doses led to a more substantial reduction in parasite density. The inhibition of *T. brucei brucei* by lemon grass extract may be attributed to its content of saponins, flavonoids, tannins, and alkaloids,<sup>24</sup> which are known to inhibit cytochrome oxidase activity in the trypanosome parasite, thus impairing the use of DNA as a template. This finding is consistent with previous studies reported by Bohui *et al.*,<sup>25</sup> For the prophylactic effect, no parasitaemia was observed at early exposure, but higher levels were recorded with the ethanolic extract at prolonged exposure, as presented in Figure 3. ANOVA revealed a three-way interaction between the variables on parasitaemia levels ( $F(12, 47) = 2.96, P = 0.0038$ ), with the Bonferroni test indicating significantly higher parasitaemia with the ethanol extract at higher dosages and 4 days post-treatment ( $p < 0.05$ ).



**Figure 1:** Parasitaemia level of Swiss albino mice treated with aqueous and methanol extracts of *Cymbopogon citratus* for 48hours (Suppressive)



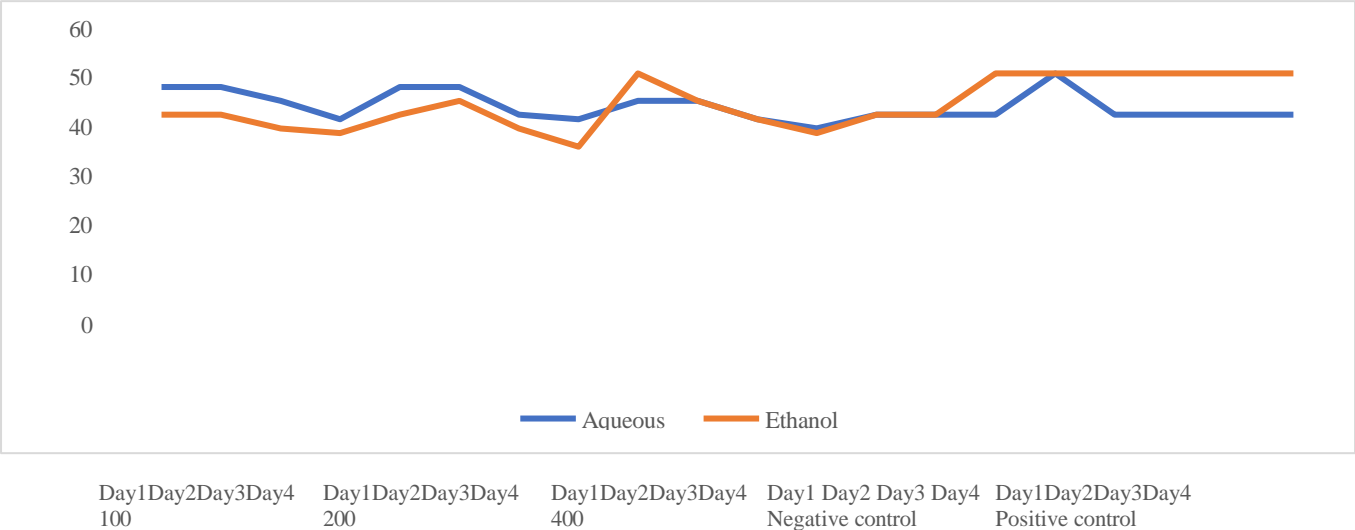
**Figure 2:** Parasitaemia level of Swiss albino mice treated with aqueous and methanol extracts of *Cymbopogon citratus* for 48 hours (Curative)



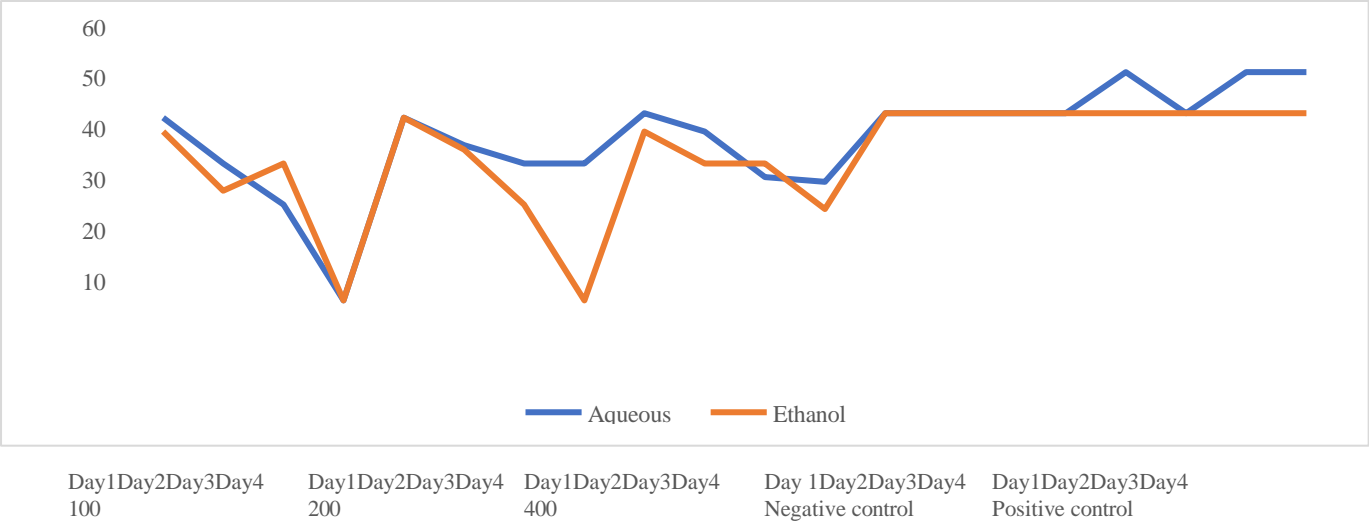
**Figure 3:** Parasitaemia level of Swiss albino mice treated with aqueous and methanol extracts of *Cymbopogon citratus* for 48 hours (Prophylactic)

The extracts' suppressive effect on PCV levels showed a slight increase with higher concentrations for the aqueous extract but a decrease for the ethanol extract, as shown in Figure 4. Both extracts caused a reduction in PCV over time, regardless of concentration. ANOVA revealed a significant interaction between solvent type and concentration on PCV levels ( $F(4, 48) = 6.12, P = 0.0005$ ). Bonferroni tests showed significantly higher PCV in the positive control for aqueous extracts and the negative control for ethanol extract ( $p < 0.05$ ). Additionally, the curative effect of PCV declined with prolonged exposure, although control groups maintained similar PCV values throughout all periods, as shown in Figure 5. At lower concentrations, PCV values were lower than at higher concentrations. ANOVA revealed no interaction between the variables; however, significant effects were observed for concentration ( $F(5, 30) = 14.49, P = 0.0000$ ) and exposure time ( $F(3, 30) = 6.15, P = 0.0022$ ). Some animals died after extended exposure. Bonferroni tests showed significantly higher PCV in control groups and on day 1 of treatment compared to later days ( $p < 0.05$ ). For the

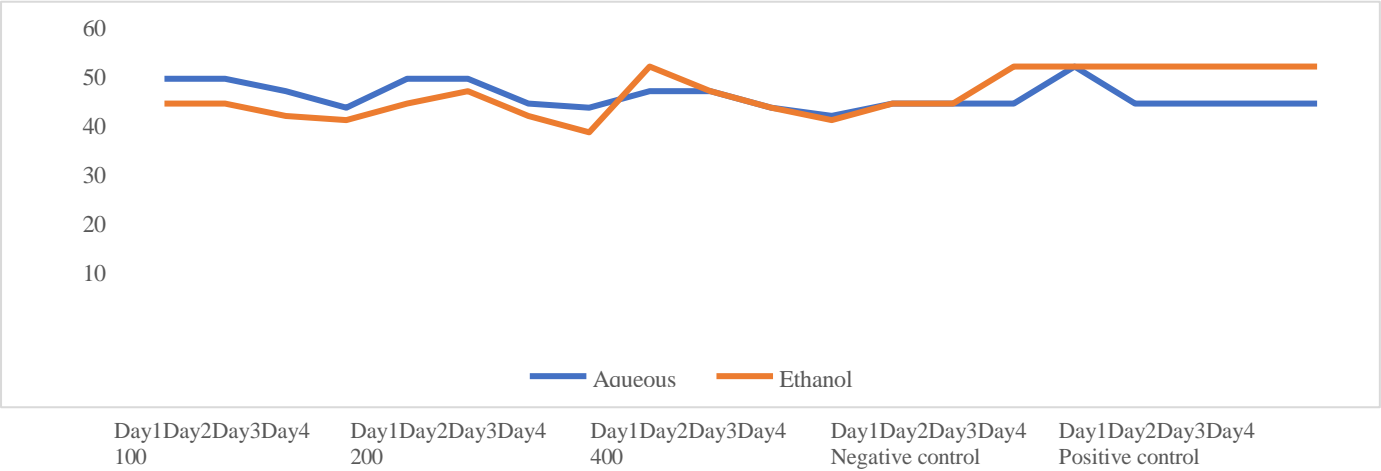
prophylactic effect, PCV decreased over time with both treatments, as shown in Figure 6. At higher concentrations, the aqueous extract caused a decline in PCV, whereas the ethanol extract led to an increase. ANOVA analysis revealed a significant interaction between concentration and solvent type ( $F(3, 48) = 3.56, P = 0.0209$ ). Bonferroni tests showed that the ethanol extract had a significantly more potent effect on PCV than the aqueous extract at the same concentration ( $p < 0.05$ ). It has been documented that trypanosome infection affects the red blood cells of an infected animal, resulting in significant changes in packed cell volume (PCV).<sup>26</sup> Parasites invade and destroy red blood cells, leading to a reduction in the number of circulating erythrocytes. This directly causes a decrease in PCV.<sup>26, 27</sup> A significant decrease in packed cell volume (PCV) was recorded in both extracts over a long-time exposure, findings consistent with the documented high pathogenicity of *Trypanosoma brucei brucei* to mice have also been reported by Anyogu *et al.*,<sup>28</sup>



**Figure 4:** Mean PCV level of Swiss albino mice treated with aqueous and ethanol extracts of *Cymbopogon citratus* for 48 hours (Suppressive).



**Figure 5:** Mean PCV level of Swiss albino mice treated with aqueous and ethanol extracts of *Cymbopogon citratus* for 48 hours (Curative).

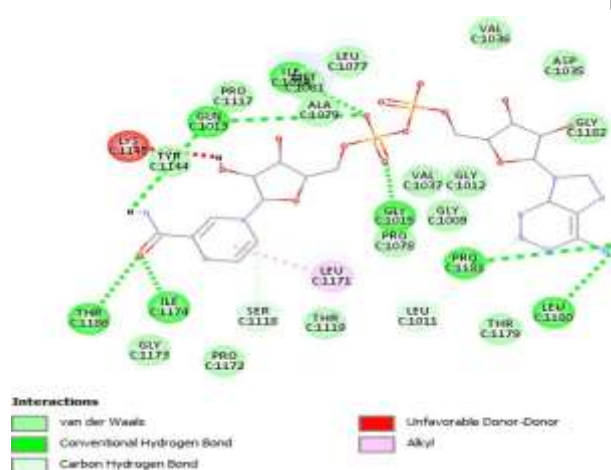


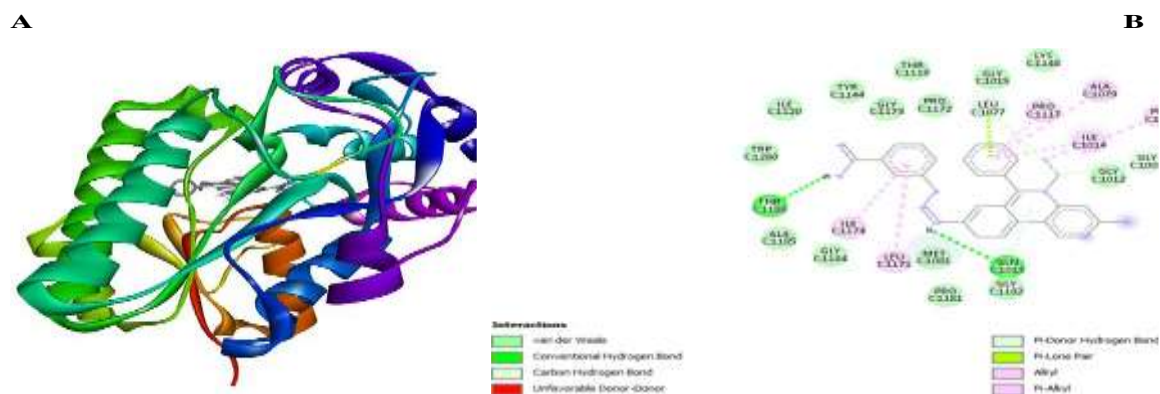
**Figure 6:** Mean PCV level of Swiss albino mice treated with aqueous and ethanol extracts of *Cymbopogon citratus* for 48 hours (Prophylactic).

from the amino acid.<sup>30</sup> In *T. brucei brucei*, this enzyme is part of the organism's ability to utilize various amino acids for energy and other cellular functions, and it can be a potential target for drug development, as interfering with such metabolic pathways could disrupt the parasite's survival.<sup>29</sup> Isometamidium chloride is thought to inhibit *T. brucei brucei* L-threonine-3-dehydrogenase by disrupting the enzyme's ability to oxidize L-threonine, thereby impairing the parasite's metabolism. The drug likely interferes with the binding of essential cofactors, such as NAD<sup>+</sup> or NADH, or obstructs the enzyme's catalytic process. This inhibition prevents the enzyme from effectively catalyzing the dehydrogenation of L-threonine, a critical step for the parasite's survival. Given the similarity in the mechanisms of action of various anti-parasitic drugs targeting *T. brucei*, Isometamidium chloride likely disrupts these essential metabolic pathways, further compromising the parasite's viability.<sup>31</sup> The Geranyl acetate, a compound from *C. citratus*, interacted with key catalytic amino acid residues THR 1186 on the *T. brucei brucei* L-threonine-3-dehydrogenase site, similar to the substrate (NAD<sup>+</sup>) and standard inhibitor (Isometamidium chloride). The interaction of Geranyl acetate with THR 1186 was through an H-bond interaction, which could form a stable ligand-enzyme complex.<sup>16</sup> Isometamidium chloride and Geranyl acetate interaction with THR 1186 might reduce the activities of *T. brucei brucei* L-threonine-3-dehydrogenase. This finding is supported by Saeidnia and Gohari, who reported the antiprotozoal effect of geranyl acetate and related monoterpenes against *Trypanosoma* species.<sup>32</sup>

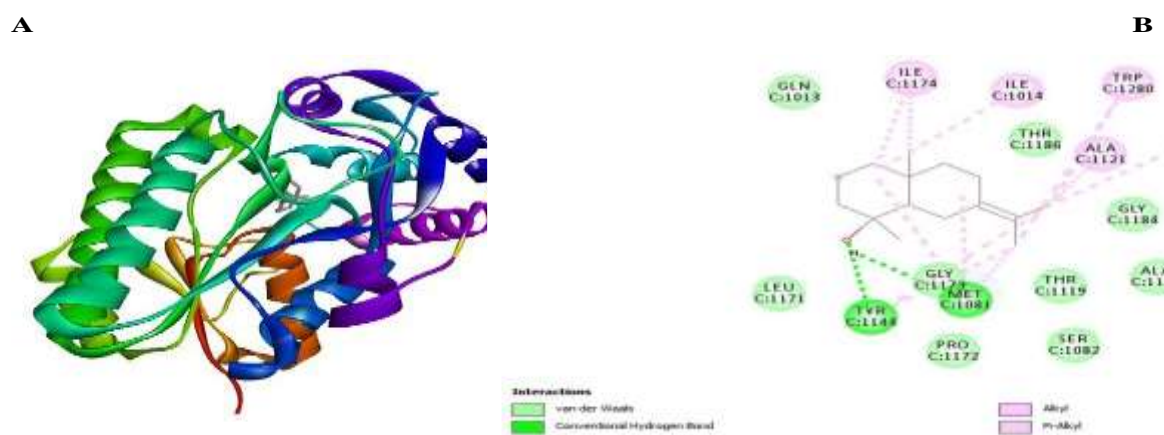
S/n	Names	Compound ID	Binding Energy (Kcal/mol)
	Standard		
1	NAD	5892	-10
2	IsometamidiumChloride	72452	-9.3
	<i>C.citratus</i> compounds		
3	Junipercamphor	521214	-6.6
4	Geranyl acetate	1549026	-6.4
5	1,2-Benzendicarboxylic acid	1017	-6.3
6	Z-Citral	638011	-5.5
7	Trans-geraniol	637566	-5.3
8	G-methyl-5-hepten-2-0ne	9862	-5.1

B

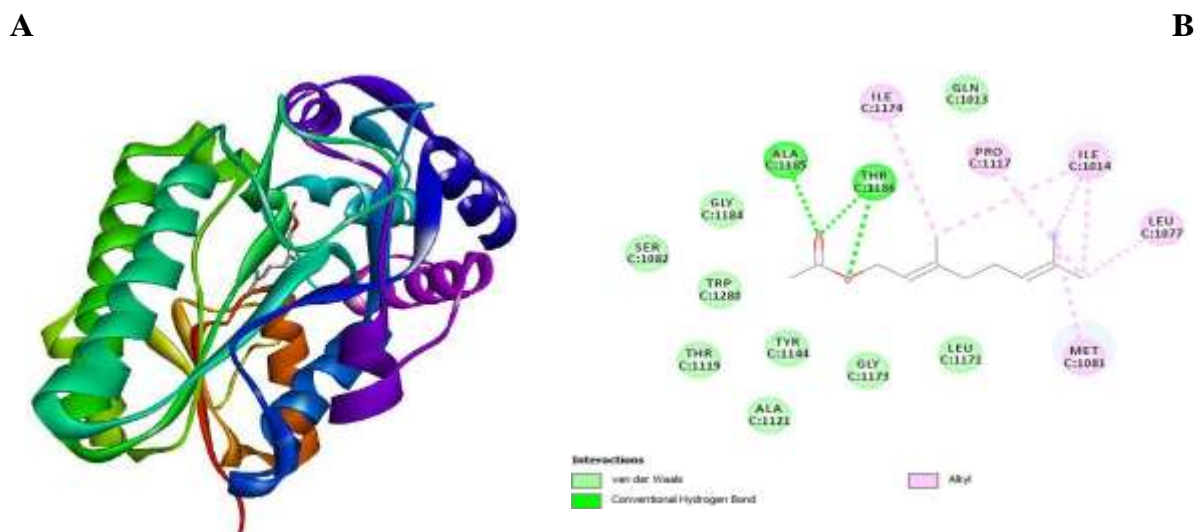




**Figure 8:** 3D (A) and 2D (B) of Molecular Interaction of Trypanosoma L-threonine-3- dehydrogenase Amino Acid Residues with Isometamidium Chloride



**Figure 9:** 3D (A) and 2D (B) of Molecular Interaction of Trypanosoma L-threonine-3- dehydrogenase Amino Acid Residues with Juniper camphor



**Figure 10:** 3D (A) and 2D (B) of Molecular Interaction of Trypanosoma L-threonine-3- dehydrogenase Amino Acid Residues with Geranyl acetate

## Conclusion

In conclusion, this study demonstrates the significant anti-trypanosomal potential of *C. citratus* leaf extracts, highlighting its efficacy in reducing parasitemia and influencing immune parameters in in-vivo models. The phytochemical analysis and molecular docking studies suggest that the bioactive compounds, particularly Geranyl acetate, may effectively inhibit *T. brucei* survival by targeting key enzymes. The promising results from both ethanol and aqueous extracts, with the ethanol extract showing superior efficacy, emphasize the potential of *C. citratus* as a valuable source for developing new, cost-effective treatments for African trypanosomiasis. These findings contribute to the growing body of research aimed at addressing the challenges posed by drug resistance and the absence of a vaccine for this debilitating disease.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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