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Phytochemistry, *In vitro* Anti-Trypanosomal Efficacy and Acute Toxicity of *Allium sativum* Linn. Bulb Aqueous Extract

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ABSTRACT

Allium sativum Linn. is a bulbous perennial plant of the Family *Liliaceae* has been reported as having potential medicinal properties. This study was on the phytochemistry, *in vitro* anti-trypanosomal efficacy and acute toxicity of *Allium sativum* Linn bulb aqueous extract using standard procedures. Qualitative phytochemistry indicated high scores (+++) for reducing sugars, moderate scores (++) for alkaloids, steroids and carbohydrates with low scores (+) for tannins, flavonoids and saponins. The median lethal dose of the aqueous extract in albino rats was determined to be 2400 mg/kg body weight following intraperitoneal administration. The albino rats exhibited weakness, lordosis, starry hair coat, awkward posture, loss of appetite. All the albino rats died within 24 hours. There was a significant ($p < 0.05$) reduction in mean parasites count ($\times 10^6$) on exposure *in vitro* to all the graded extract concentrations compared with the normal, and parasite count also decreased with time post-inoculation. Similarly, a positive correlation existed between extract concentrations and inhibition rates of the parasites post-inoculation. In conclusion, the aqueous extract of *Allium sativum* has *in vitro* anti-trypanosomal activity.

Keywords: Acute Toxicity; *Allium sativum*; Anti-Trypanosomal efficacy; Phytochemistry

INTRODUCTION

Garlic (*Allium sativum* L.) is a member of the *Alliaceae* Family and, after onions, is the second most widely used *Allium* species. It is produced and used globally as a spice, additive, and medicine for the popular treatment of a variety of diseases and physiological conditions (Onyeagba *et al.*, 2004; Tesfaye and Mengesha, 2015). Garlic has a higher concentration of sulfur compounds (allicin, diallyl disulfide, S-allylcysteine, and diallyl trisulfide), which are responsible for its therapeutic properties. As a result, scientists in numerous fields are currently concentrating their efforts on determining the medicinal potential of garlic in human health (Chan *et al.*, 2013; Bayan *et al.*, 2014). There are several reports on the enormous set back effects of trypanosomiasis on human and livestock productivity in Africa. Natural plant products offer novel opportunities for trypanocides, and *Allium sativum*, widely distributed in Nigeria, could be one of them due to its several pharmacological activities against a vast range of blood and intestinal parasites (Hoet *et al.*, 2004; Bora and Sharma, 2009; Kahrizi *et al.*, 2012; Mikaili, *et al.*, 2013; Elberry *et al.*, 2014; Alagarsamy, 2018; Biu *et al.*, 2022).

MATERIALS AND METHODS**Preparation of Aqueous Extract**

Fresh *Allium sativum* bulbs were purchased from Gaboru vegetable market Maiduguri, and authenticated in the botany unit herbarium of the Department of Biological Sciences, University of Maiduguri, Nigeria, where a voucher specimen was deposited. These bulbs were peeled and rinsed in clean water, sliced into pieces and air dried under laboratory conditions avoiding solar leaching for a period of 4 weeks, then ground into fine powder using pestle and mortar to obtain a 1000g weight. The cold aqueous percolation extraction method was used as described by ICS-UNIDO, (2008) and Obeidat *et al.*, (2012) and gave a yield of 8.13%.

Phytochemical Screening

Allium sativum aqueous extract was qualitatively screened for bioactive substances namely reducing sugars, alkaloids, steroids, carbohydrates, tannins, flavonoids, saponins and anthraquinones using standard biochemical procedures of Evans, (2002) and Parekh and Chanda, (2007) and observations made on reagent test colour changes.

Acute Toxicity Testing

Twenty one albino rats of both sexes weighing between 72.3 and 96.3 grams acquired from the animal breeding house, University of Maiduguri were divided into 7 groups, of 3 rats each (A-G). They were kept in polyacrylic cages and fed with growers mash (Ultima Feed Plc, Nigeria) and clean drinking water *ad libitum*.

Groups A – F were administered intraperitoneally with graded doses of 100, 200, 400, 800, 1600, and 3200 mg/kg/body weight of *Allium sativum* aqueous extract and observed for clinical signs of toxicity and the LD₅₀ calculated using the modified Arithmetic method of Karber (Aliu and Nwude, 1982.) while group G served as normal control. Use of albino rats were according to international guidelines on animal welfare as described by Demers *et al.*, (2006).

In vitro-Testing

The protocols of Atawodi *et al.*, (2003) were adopted by weighing 200mg of the *Allium sativum* aqueous extract dissolved in 5mls of phosphate buffered saline solution (PBSS) giving 40mg/ml concentration, followed by serial dilution of the stock using PBSS to obtain descending grades of 40.0, 20.0, 10.0, 5.0, 2.5, 1.25, 0.625, 0.313, 0.156 and 0.078 mg concentrations. *Trypanosoma brucei brucei* Federe strain/NTR/14 obtained from the Nigerian Institute for Trypanosomiasis and Onchocerciasis Research (NITOR) Vom, Plateau State, Nigeria, stabilized through serial passage in donor albino rats and identified based on their morphology and negative blood inhibition infectivity test (BIT).

Evaluation of the *in vitro* anti-trypanosomal efficacy was done using test tubes where 2 μ l of *T. b. brucei* infected albino rat tail blood and 1ml of phosphate buffered glucose saline solution (PBGSS) was inoculated into each test tube

replicated 8 times containing each graded *Allium sativum* aqueous extract concentration and incubated at 37^o C in a water bath for 15 minutes. The improved Neubauer's chamber was used to count trypanosomes per field under the light microscope (x40 magnification) at an interval of 30, 60, 90 and 120 minutes.

Two control groups also of 8 replicates each made up of negative control inoculated with 2 μ l of infected blood and 1ml of PBGSS and a positive control, also with 2 μ l of blood, 1ml PBGSS and treated with 1ml of 3.5mg Veriben^R (Diminazine acetate, Eagle Chemical Company Ltd., Ikeja, Lagos, Nigeria). Parasite count was expressed as mean \pm standard deviation (SD) $\times 10^6$ /ml and percentage inhibition against time post-inoculation (minutes) with statistical analysis using the "t" test at p = 0.05 (SPSS Version 16, 2011).

Ethical Statement

This work was conducted in line with the guidelines for animal care and use.

Data Analyses

Data obtained from this study were analysed by Graph Pad Instat Biostatistic Version 3 Inc®, U.S.A., 2002 software using one way analysis of variance (ANOVA) where values are expressed as Mean \pm SD and p value \leq 0.05 were considered significant

RESULTS

Table 1 in this study shows that reducing sugars had high scores, alkaloids, steroids and carbohydrates moderate scores, tannins, flavonoids and saponins low scores and also the detailed observations on reagent test changes

Table 1: Qualitative Phytochemistry of the Aqueous Extract of the Bulb of *Allium sativum* Linn

| Constituent | Tests | Observations | Inferences |
|-----------------|-----------------------|------------------------|------------|
| Alkaloids | Dragendorff's | Orange red precipitate | ++ |
| Flavonoids | Shinoda's | Rose red | + |
| Anthraquinones | Borntrager's | None | - |
| Steroids | Liebermann-Burchard's | Bluish-green | ++ |
| Saponins | Frothing | Persisted foam column | + |
| Reducing sugars | Fehling's | Reddish brown | +++ |
| Carbohydrates | Molisch's | Purple | ++ |
| Tannins | Ferric chloride | Blue black green | + |

Key: +++ = High concentration, ++ = Moderate concentration, + = Low concentration, - = Not detected

The *in vitro* anti-trypanosomal testing in this study shows the mean \pm SD (range) parasite count ($\times 10^6$ /ml) time post-inoculation (minutes) (Table 2) and parasite inhibition rate (Table 3). The extract concentration 1.25mg at 120 minutes

post-inoculation had the lowest mean parasite count and the highest % inhibition, indicating a positive relationship between extract concentration, mean count and inhibition rate of *Trypanosoma brucei brucei* and minutes post-exposure

Table 2: In Vitro Effect of Graded Concentrations of *Allium sativum* Bulb Aqueous Extract *Trypanosoma brucei brucei* Count

| Extract concentration (mg/ml) | Mean \pm SD Parasite Count/ml ($\times 10^6$) Time Post Inoculation (Minutes) | | | |
|-------------------------------|--|------------------------------|-------------------------------|-------------------------------|
| | 30 | 60 | 90 | 120 |
| PBGSS (Control) | 3.20 \pm 0.05 ^a | 3.20 \pm 0.08 ^a | 3.20 \pm 0.10 ^a | 3.20 \pm 0.08 ^a |
| 0.078 | 1.05 \pm 0.08 ^b | 0.96 \pm 0.06 ^b | 0.81 \pm 0.06 ^b | 0.77 \pm 0.08 ^b |
| 0.156 | 0.85 \pm 0.02 ^b | 0.73 \pm 0.05 ^c | 0.65 \pm 0.05 ^b | 0.39 \pm 0.04 ^c |
| 0.313 | 0.68 \pm 0.05 ^c | 0.57 \pm 0.05 ^c | 0.40 \pm 0.01 ^c | 0.29 \pm 0.02 ^{cd} |
| 0.625 | 0.35 \pm 0.05 ^d | 0.29 \pm 0.04 ^d | 0.22 \pm 0.02 ^{cd} | 0.11 \pm 0.07 ^d |
| 1.25 | 0.81 \pm 0.05 ^b | 0.10 \pm 0.03 ^d | 0.06 \pm 0.02 ^d | 0.07 \pm 0.01 ^d |
| 2.5 | 00 \pm 00 ^e | 00 \pm 00 ^e | 00 \pm 00 ^e | 00 \pm 00 ^e |
| 5.0 | 00 \pm 00 ^e | 00 \pm 00 ^e | 00 \pm 00 ^e | 00 \pm 00 ^e |

PBGSS=Phosphate buffered glucose saline solution. ^{abcd}Mean values with different superscripts between are significantly different (p<0.05).

Table 3: Parasite Inhibition (%) Time Post- Inoculation (Minutes)

| Extract Concentration (mg/ml) | % Inhibition | | | |
|-------------------------------|---------------------------------|-------------------|--------------------|--------------------|
| | Time Post-Inoculation (Minutes) | | | |
| | 30 | 60 | 90 | 120 |
| PBGSS (Control) | 00 ^a | 00 ^a | 00 ^a | 00 ^a |
| 0.078 | 67.8 ^b | 69.8 ^b | 67.9 ^b | 67.7 ^b |
| 0.156 | 73.7 ^b | 75.5 ^b | 80.0 ^c | 88.2 ^c |
| 0.313 | 79.8 ^c | 82.4 ^c | 89.1 ^d | 92.1 ^{cd} |
| 0.625 | 89.8 ^d | 90.9 ^d | 93.8 ^{de} | 95.9 ^d |
| 1.25 | 95.9 ^{de} | 99.4 ^e | 99.9 ^e | 100 ^d |
| 2.5 | 100 ^e | 100 ^e | 100 ^e | 100 ^d |
| 5.0 | 100 ^e | 100 ^e | 100 ^e | 100 ^d |
| 10.0 | 100 ^e | 100 ^e | 100 ^e | 100 ^d |
| 20.0 | 100 ^e | 100 ^e | 100 ^e | 100 ^d |
| 40.0 | 100 ^e | 100 ^e | 100 ^e | 100 ^d |

Values with different superscripts between columns are statistically significant ($p < 0.05$).

The median lethal dose (LD₅₀) is shown in Table 4. The extract at the dose of 3200mg/kg/ body weight produced a 100% mortality while the LD₅₀ was 2400mg/kg/body weight

no mortality was observed at graded concentrations of 100, 200, 400, 800, and 1600 mg/kg/body weight.

Table 4: Median Lethal Dose (LD₅₀) of *Allium sativum* Aqueous Extract in Albino Rats

| Groups (n=3) | Extract Concentrations (mg/kg) | Dose Difference (DD) | Number Dead | Mean Dead (MD) | DD × MD |
|--------------|--------------------------------|----------------------|-------------|----------------|---------|
| A | 100 | | 0 | | |
| B | 200 | B – A = 100 | 0 | 0 | 0 |
| C | 400 | C – B = 200 | 0 | | 0 |
| D | 800 | D – C = 400 | 0 | 0 | 0 |
| E | 1600 | E – D = 800 | 0 | | |
| F | 3200 | F – E = 1600 | 3 | 1.5 | 2400 |
| G | Normal Control | - | - | - | - |
| Total | | | | | 2400 |

$$LD_{50} = LD_{100} - \frac{DD \times MD}{n} = 3200 - \frac{2400}{3} = 2400 \text{ mg/kg/ body weight}$$

DISCUSSIONS

In this study, reducing sugars had high scores, alkaloids, steroids and carbohydrates moderate scores, tannins, flavonoids and saponins low scores. These findings are similar to those identified by Garba *et al.*, (2013) Huzaiifa *et al.*, (2014) and Biu, *et al.*, (2022) however slight differences with this study could be attributed to environmental differences (Stein *et al.*, 2017). Plants of the Family *Liliaceae* are reported to be very rich in phytoconstituents that add value to their medicinal and economic status as herbal products (Bora and Sharma 2009). The median lethal dose (LD₅₀) was 2400mg/kg/body weight in this study, which according to the toxicity scale of Hodge and Sterner, (2005) classified as “slight” has been evidenced by the zero mortality at doses lower than 3200mg/kg/body weight.

The *in vitro* anti-trypanosomal testing in this study has shown the lowest mean ± SD parasite count ($\times 10^6$ /ml) and a 100% parasite inhibition rate at the extract concentration of 1.25mg at 120 minutes post-inoculation indicating a positive relationship between extract concentration, mean parasite count, inhibition rate and minutes post- exposure of *Trypanosoma brucei brucei*. The oil extract of *Allium sativum* has been reported to inhibit phospholipidase activity in *Trypanosoma congolense* and natural plant products are capable of peroxidative damage to the trypanosothione reductase in trypanosomes altering their redox balance, and garlic is said to contain sulphur compounds that destroys mitochondrial function in trypanosomes (Kristin *et al.*, 2018). The various phytoconstituents of *Allium sativum* in this study

might thus be responsible for its anti-trypanosomal effects of inhibition or mortality.

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Authors Contribution

LBB and AAB conceived, designed and coordinated the story. SY, UU and AAK conducted the experiments. UAM participated in data analysis, interpretation and drafting and submitting the manuscript for publication.

Conflict of Interest

The authors have no conflict of interest to declare

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