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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 bulbos 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, steriods 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 apetite. All the albino rats died within 24 hours. There was a significant ($p<0.05$) reduction in mean parasites count ($x10^6$) 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 trypanasomosis 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 Gamboru 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 yeild 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 devided 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 LD_{50} calculated using the modified Arithmetic method of Karber (Aliu and Nwude, 1982.) while group G served as nornal 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* aqeuoes extract disolved 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. *Trypanasoma 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 2µl 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 37^0 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 2µl of infected blood and 1ml of PBGSS and a positive control, also with 2µl of blood ,1ml PBGSS and treated with 1ml of 3.5mg Veriben^R (Diminazine aceturate, Eagle Chemical Company Ltd., Ikeja, Lagos, Nigeria). Parasite count was expressed as mean ± standard deviation (SD) x 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 ± SD and p value ≤ 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 ± SD (range) parasite count (x 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 ± SD Parasite Count/ml (x 10^6) Time Post Inoculation (Minutes)			
	30	60	90	120
PBGSS (Control)	3.20±0.05^a	3.20±0.08^a	3.20±0.10^a	3.20±0.08^a
0.078	1.05±0.08^b	0.96±0.06^b	0.81±0.06^b	0.77±0.08^b
0.156	0.85±0.02^b	0.73±0.05^c	0.65±0.05^b	0.39±0.04^c
0.313	0.68±0.05^c	0.57±0.05^c	0.40±0.01^c	0.29±0.02^{cd}
0.625	0.35±0.05^d	0.29±0.04^d	0.22±0.02^{cd}	0.11±0.07^d
1.25	0.81±0.05^b	0.10±0.03^d	0.06±0.02^d	0.07±0.01^d
2.5	00±00^e	00±00^e	00±00^e	00±00^e
5.0	00±00^e	00±00^e	00±00^e	00±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 (LD_{50}) is showm in Table 4. was The extract at the dose of 3200mg/kg/ body weight produced a 100% mortality while the LD_{50} 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 (LD_{50}) 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

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

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) Huzaifa *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 (LD_{50}) was 2400mg/kg/body weight in this study, which according to the toxicity scale of Hodge and Sterner, (2005) classifified 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 (x 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 *Trypanasoma 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

REFERENCES

Alagarsamy, S., Chellappan, P., Jesuraj, M. T., Mohan, M. S. G. and Balakrishnan, R. (2018). Phytochemical analysis and antioxidant potential of the crude extract of Allium oschaninii scape. *Oriental Pharmacy and Experimental Medicine, 18, 309-316.* Doi: https://doi.org/10.1007/s13596-018-0339-5,

Aliu, Y. O. and Nwude, N. (1982). *Veterinary Pharmacology and Toxicology Experiments*. Ahmadu Bello University Press, Zaria, pp. 104-110.

Atawodi, S. E., Bulus, T., Ibrahim, S., Ameh, D. A., Nok, A. J., Mamman, M. and Galadima, M. (2003). *In vitro* trypanocidal effect of methanolic extract of some Nigerian savannah plants. *African Journal*

of Biotechnology, 2(9), 317-321.DOI:10.5897/AJB2003.000-1065,

Bayan L., Koulivand P. H. and Gorji A. (2014). Garlic: A review of potential therapeutic effects. *Avicenn Journal of Phytomedicine* . 2014; 4(1):1–14.

Biu, A. A., Auwal, M. S., Maina, U. A., Salihu, S. I., Kashimbe, I. B., Buratai, L. B., Ibrahim, A., Lawan, F.A. and Ruth, P. A. (2022). *In vitro* Trypanocidal Activity of Aqueous Leaves Extract of *Allium cepa* linn in Experimentally Infected Wistar Rats. *Sahel Journal of Veterinary Sciences,* 19(2): 1-6. Doi https://doi.org/10.54058/saheljvs.v19i2.295

Bora, K., and Sharma, A. (2009). Phytoconstituents and therapeutic potential of *Allium cepa* Linn. *A review. Pharmacognosy Reviews*, 3(5), 170-180.

Chan, R. Y.K. and Chan, S.W. A review of the cardiovascular benefits and antioxidant properties of allicin*. Phytot herapy Research*. 2013; 27(5):637-646. https://doi.org/10.1002/ptr.4796,

Demers, G., Griffin, G., De Vroey, G., Haywood, J. R., Zurlo, J. and Bédard, M. (2006). *Harmonization of animal care and use guidance*. *Science*, 312 (5774), 700-701. DOI: 10.1126/science.1124036,

Elberry, A. A., Mufti, S., Al-Maghrabi, J., Abdel Sattar, E., Ghareib, S. A., Mosli, H. A. and Gabr, S. A. (2014). Immunomodulatory effect of red onion (*Allium cepa* Linn.) scale extract on experimentally induced atypical prostatic hyperplasia in Wistar rats. *Mediators of Inflammation,* 2014. Doi; https://doi.org/10.1155/2014/640746,

Evans, W. C. (2002) *Textbook of Pharmacognosy*, 14th edition. Beithere Tindal, W. B. Saunders Co. Ltd. Engalnd, Pp. 117 – 575.

Garba, I., Umar, A. I., Abdulrahman, A. B., Tijjani, M. B., Aliyu, M. S., Zango, U. U. and Muhammed, A. (2013). Phytochemical and antibacterial properties of garlic extract. *Bayero Journal of Pure and Applied Sciences,* 6(2):45-48. Doi; https://doi.org/10.4314/bajopas.v6i2.10,

Hodge, A. and Sterner, B. (2005). Toxicity classes. In: Canadian center for occupational Health and safety. https://www.ccohs.ca/oshanswers/chemicals/ld50.html on 14th January 2019.

Hoet, S., Opperdoes, F., Brun, R. and Quetin-Leclercq, J. (2004). Natural products active against African trypanosomes: a step towards new drugs. *Natural Product Reports, 21(3), 353-364.* Doi; https://doi.org/10.1039/B311021B

Huzaifa, U., Labaran, I., Bello, A.B. and Olatunde, A. (2014). Phytochemical screening of aqueous extract of garlic (*Allium sativum*) bulbs. *Report and Opinion*. 6(8):1-4.

International Center for Scientific and High Technology-United Nations Industrial Development Organisation (ICS - UNIDO) (2008). Extraction Technologies for Medicinal and Aromatic Plants. Handa, S.S., Khanuja, S.P.S., Lango, G., Rokesh, D. D. Eds. ICS-UNIDO Area Park, Padriciano 99, 34012 Trieste Italy.

Kahrizi, D., Molsaghi, M., Faramarzi, A., Yari, K., Kazemi, E., Farhadzadeh, A. M., and Yousofvand, N. (2012). Medicinal plants in holy Quran. *American Journal of Scientific Research,* 42(42), 62-71.

Krstin, S., Sobeh, M., Braun, M.S. and Wink, M. (2018). Anti-Parasitic activities of *Allium sativum* and *Allium cepa* against *Trypanosoma b. brucei* and *Leishmania tarentolae.Medicines* (Basel) 5(2): pp 37. Doi:10.3390/medicines5020037.

Mikaili, P., Maadirad, S., Moloudizargari, M., Aghajanshakeri, S. and Sarahroodi, S. (2013).Therapeutic Uses and Pharmacological Properties of Garlic, Shallot, and Their Biologically Active Compounds*. Iranian Journal of Basic Medical Science*. 16(10): 1031–1048.

Obeidat, M., Shatnawi, M., Al-alawi, M., Al-Zu`bi, E., Al-Dmoor, H., Al-Qudah, M., El-Qudah, J. and Otri, I. (2012). Antimicrobial activity of crude extracts of some plant leaves. *Research Journal of Microbiology*, 7: 59-67. Doi;10.3923/jm2012.59.65,

Onyeagba R. A., Ugbogu O. C., Okeke C. U. and Iroakasi O. Studies on the antimicrobial effects of garlic (*Allium sativum* Linn.), ginger (*Zingiber officinale* Roscoe) and lime (*Citrus aurantifolia* Linn). *African Journal of Biotechnology. 2004; 3(10):552-554. doi: 10.5897/ajb2004.000-2108,*

Parekh, J. and Chanda S. V. (2007). *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal Plants. *Turk Journal of Biology*, Pp. 31-53. https://journals.tubitak.gov.tr/biology/vol31/iss1/9

SPSS version 16. Statistical Package for Social Sciences 2011, version 20.0. IBM Corporation, New York, USA

Stein, R. J., Höreth, S., De Melo, J.M.F, Syllwasschy, L., Lee, G., Garbin, M. L., Stephan Clemens, S. and Ute Krämer, U. (2017). Relationships between soil and leaf mineral composition are element-specific, environment-dependent and geographically structured in the emerging model Arabidopsis halleri. *The New Phytologist*. 213(3): 1274–1286. doi: 10.1111/nph.14219

Tesfaye A., Mengesha W. (2015). Traditional uses phytochemistry and pharmacological properties of garlic (*Allium Sativum*) and its biological active compounds. *International Journal of Sciences Research Engineering and Technology.* 2015; 1:142–148. Doi; https://doi.org/10.3390/nu12030872,