https://dx.doi.org/10.4314/jpb.v18i3.1
Vol. 18 no. 3, pp. 172-181 (September 2021)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND
BIORESOURCES

Anti-trypanosomal, antioxidant and antimicrobial activities of the fruiting bodies of *Ganoderma lucidum* (W. Curt.: Fr) (Ganodermataceae) aqueous extract

Samuel Otokpa EDE[*1], John Chinyere AGUIYI[1], Simeon OMALE[1], Rebecca Folashade EDE[2]

[1]*Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences;* [2]*Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, College of Health Sciences, University of Jos, P.M.B. 2084, Jos. Nigeria.*

Received 13th January 2021; Accepted 3rd August 2021

Abstract
Human African Trypanosomiasis (HAT) infection is caused by sub species of *Trypanosoma brucei*. The clinically licensed drugs have unacceptable toxicities and variable efficacies. The prognosis is influenced by the level of circulating free radicals and opportunistic infections. Many plants have been screened for activity in different models of HAT but the same is not true of mushrooms. This study screened the aqueous extract of the fruiting bodies of *Ganoderma lucidum* for trypanocidal, antioxidant and antimicrobial activities. Fruiting bodies of the mushroom were extracted sequentially with n – hexane, ethyl acetate, absolute ethanol and distilled water. The extracts were screened for phytochemical constituents and *in vitro* trypanocidal activity, the most active of which was further subjected to *in vivo* trypanocidal, anti-oxidant and anti-microbial screening. Alkaloids, carbohydrates, flavonoids, saponins and anthraquinones were present. *In vitro* trypanocidal screening showed the aqueous extract as the most active (IC_{50} = 14.65 µg/µl). It also dose-dependently inhibited parasitaemia and prolonged survival in parasite - infected mice (50, 100, 200 and 400 mg/kg). DPPH radical scavenging activity gave an IC_{50} of 131.00 ± 0.03 mg/ml. The extract demonstrated broad spectrum antimicrobial activity at 250 mg/ml. The fruiting bodies of *G. lucidum* is a potential source of trypanocidal compounds.

Keywords: *Ganoderma lucidum*; Mushrooms, Trypanocidal; *Trypanosoma brucei brucei*,

INTRODUCTION

Human African trypanosomiasis (HAT, sleeping sickness) is a parasitic disease caused by sub - species of the haemoflagellate *Trypanosoma brucei*, namely *Trypanosoma brucei gambiense* (west African trypanosomiasis) and *Trypanosoma brucei rhodensiense* (East African trypanosomiasis) [1], transmitted by haemophageous tsetse flies [2]. It is a neglected tropical disease (NTD) which affects nearly 30 million people in sub – Saharan Africa [3]. The infection causes a wide range of peripheral and central symptoms including anaemia, obtundation due in part to trypanosome – induced oxidative stress and secondary microbial infections [4,5].

Chemotherapy, with synthetic drugs, is the main stay of management using the early

*Correspondence. *E-mail*: samotkede@yahoo.com *Tel*: +234-7030376867. ISSN 0189-8442

phase drugs suramin and pentamidine, and the latter phase drug melarsoprol. However, increasing reports of drug resistance, toxicities, complex administration regimen and variable efficacies of these drugs create an urgent need for medicines with better safety profile and efficacy [6,7]. Unfortunately, the resources needed for the development of newer chemotherapeutic agents against this disease are scarce, largely because the resource constraints of the affected population do not provide guaranteed returns on any investments made in the drug discovery process [8]. Most patients therefore have to resort to traditional remedies [9]. It is well documented that traditional medicine is a vital part of the health care delivery system for many African communities [10] and a first resort before orthodox treatment is sought.

Many plants are used indigenously for the traditional management of HAT and a good number have been scientifically screened and the claims validated [1,11]. Mushrooms, like plants, are comparatively endowed with a plethora of medicinally useful bioactive constituents [12,13]. However, there are only a limited number of reports on the screening of mushrooms against trypanosomiasis. Polysaccharides from the mushroom *Lentinus strigosus* showed some activity against *Trypanosoma cruzi*, the causative organism responsible for south American trypanosomiasis (Chagas disease) [14,15], while in another study, mushroom extracts did not show any activity against *Trypanosoma congolese* [16]. In view of the foregoing, there is a need to further evaluate mushrooms for activity against *Trypanosoma brucei brucei* to clarify their potential trypanocidal effects.

Ganoderma lucidum (W. Curt.: Fr.) (Ganodermataceae) is reported to have various biological properties such as anti-oxidant, anti-microbial and anti-diabetic activities [17-20]. Similarly, different species from the genus such as *G. applanatum, G. boninense, G. colossum, G. lucidum* and *G. sinense* are reported to possess antibacterial, antifungal and antiviral properties, due to identified compounds such as colossolactone, ganoderic acid and ganoderiol [21]. However, reports on the antiparasitic property of extracts from *Ganoderma* species are limited to scanty publications on their antiplasmodial activity [21]. There are none on their trypanocidal activity. The objective of this study therefore was to screen different extracts of the fruiting bodies of *Ganoderma lucidum* (W. Curt.: Fr.) for possible trypanocidal, antimicrobial and antioxidant activities. Our experimental design was to first determine the most active of the extracts in the *in vitro* trypanocidal screening and then use this in the *in vivo* trypanocidal, anti-oxidant and anti-microbial studies. The data from this study would help the scientific community in its search for new leads against trypanosomiasis.

EXPERIMENTAL METHODS

Mushroom collection and preparation of extracts. The fruiting bodies of *Ganoderma lucidum* were collected from the wild in Kunwur village in North Central Nigeria between the months of June and September, 2013. The Samples were identified by Mr. Valentine Trevor (botanist) of the Africa Centre of Phytomedicine Research and Development, University of Jos. The fruiting bodies were cleaned of debris, dirt and shade dried over a period of 21 days and then reduced to coarse powder in a mortar and pestle. Seven hundred and fifty grams (750 g) of the coarse powder was sequentially and serially macerated in 1500 ml of cold n-hexane, ethyl acetate, absolute ethanol and distilled water, respectively for 72 h. The resulting extracts were filtered and evaporated to dryness at 40^{O}C *in vacuo*.

Animals. Thirty albino mice (22 – 25 g) of mixed sex were sourced from the Animal Experimental Unit of the University of Jos. They were kept in standard polypropylene cages (72 cm x 36 cm x 44 cm) under 12-12

hour daylight-dark cycle with access to water and food *ad libitum*. They were allowed to acclimatize for two weeks before commencement of the experiments. Animal experiments were conducted in strict accordance with institutional guidelines on animal use and welfare of the University of Jos, for which ethical approval was sought for and obtained (Ethics clearance number is F17 – 00379). The parasite used in this study was the virulent strain of *Trypanosoma brucei brucei* (Federe Strain) obtained from the Nigeria Institute for Trypanosomiasis Research, Vom Plateau State, Nigeria.

Phytochemical analyses. The extracts were evaluated for their phytochemical constituents according to standard protocols described by Sofowora [21].

Trypanocidal studies

In vitro trypanocidal screening. The different extracts were evaluated for *in vitro* trypanocidal activity in a 200 µl 96-well microtitre plate. Forty microlitres of each extract (50 µg/µl) was serially diluted across the wells with phosphate buffered saline – PBS - (NaCl 0.8, KCl 0.02, Na_2HPO_4 0.14, KH_2PO_4 0.024 % w/v) giving a concentration range of 0.97, 0.19, 0.39, 0.78, 1.56, 3.13, 6.25, 12.50, 25.00 and 50.00 µg/µl. Forty microlitres of massively parasitized blood (500.00 x 10^6 parasites per ml of blood) obtained retrobulbally from a *T. brucei brucei* infected rat was transferred into each test well and the content mixed. The positive control wells contained diminazine aceturate (0.97, 0.19, 0.39, 0.78, 1.56, 3.13, 6.25, 12.50, 25.00 and 50.00 µg/µl) The last well, which served as negative control, contained only PBS and parasitized blood. The plate was incubated at 25 ^{O}C for 10 minutes after which microscopic estimation of the parasite content of each well was done at X400 magnification. The concentration of the extract that reduced the number of motile parasites by half (IC_{50}) was determined. The most active extract from this *in vitro* test was further tested for *in vivo* trypanocidal, anti-oxidant and anti-microbial activities.

In vivo trypanocidal activity. Thirty mice were divided into 6 groups of 5 mice each representing vehicle control (group 1); treatment groups at doses of 50, 100, 200 and 400 mg/kg body weight of the aqueous extract of *G. lucidum* (groups 2 - 5) and diminazine aceturate (3.4 mg/kg) control (group 6). The mice were inoculated intraperitoneally with 50 µl of parasitized blood containing 0.50 x 10^6 parasites per ml of blood, obtained retrobulbally from a *T. brucei brucei* infected donor mice. Treatments were administered intraperitoneally as a single dose immediately parasitaemia was established, as observed microscopically. The mice were observed daily for microscopic estimation of parasitaemia by wet film preparation using a drop of caudal blood. In all cases the degree of parasitaemia was estimated and expressed as number of motile trypanosomes per ml of blood.

Antioxidant test. The 2,2-Dipheny-1-picrylhydrazyl (DPPH) radical scavenging test was conducted using protocol reported by Brand-Williams et al [22] in order to evaluate the anti – oxidant activity of the aqueous extract of *G. lucidum*. Briefly, triplicate concentrations (3.91, 7.81, 15.63, 31.25, 62.50, 125.00 mg/ml) of the aqueous extract of *G. lucidum* was prepared in methanol to yield a final volume of 3 ml to which was added 1 ml of 0.1 mM of DPPH solution in methanol. Gallic acid and rutin solutions were similarly prepared. The reaction tubes were incubated for 30 minutes, protected from light after which the absorbances of the solutions were taken at 517 nm with a UV spectrophotometer. The percentage inhibition was calculated according the formular below:

$$\text{Percentage inhibition} = [\{A_c - A_t\}/A_c] * 100$$

Where: A_c is the absorbance of the control
A_t is the absorbance of the extract

IC_{50} is the concentration of the test that produced a 50 % inhibition.

Determination of antimicrobial activity. The cup diffusion method was used to screen the effect of the aqueous extract of *G. lucidum* (62.5 – 250.0 mg/ml) against *P. aeruginosa, S. aureus, Bacillus subtilis* and *Candida albicans,* according to procedures described by Usman et al [23]. Briefly, Mueller – Hinton agar was aseptically prepared and seeded separately with the isolates. Seven millilitres was poured into pre - labeled sterilized glass petri dishes. After the agar had set, 4 holes were bored into the plates with a sterilized 9 mm cork borer. Thereafter, 0.2 ml of the extract was introduced into the wells and incubated at 37 ^{O}C for 24 hours, at the end of which the plates were examined for clear zones of growth inhibition. The experiment was carried out in triplicates. The bacterial specimen used in this study were clinically derived isolates which were identified using standard biochemical tests. Microscopy and Gram staining were first performed. *Pseudomonas aeruginosa* was confirmed by the oxidase and urase tests which identifies cytochrome oxidase producing organisms [24]. *Staphylococcus aureus* was identified using the catalase and triple sugar tests. The former is based on identifying organisms that produce the hydrogen peroxide metabolizing enzyme known as catalase [25]. Bacillus *subtilis* was confirmed using the indole test which gives a positive result with tryptophanase producing organisms [24].

Statistical analysis. Treatment groups were compared with vehicle control groups and results were analyzed by simple t – test and analysis of variance (ANOVA). Differences at $p < 0.05$ were deemed statistically significant.

RESULTS

Phytochemical screening of the extracts of *G. lucidum* showed the presence of alkaloids, carbohydrates, flavonoids, saponins and anthraquinones (Table 1). Evaluation of the *in vitro* trypanocidal activity of the different extracts of the fruiting bodies of *G. lucidum* showed that the aqueous extract demonstrated the greatest activity with an IC_{50} value of 14.65 µg/µl (Table 2) while the least active was the n – hexane extract (IC_{50} = 1500 µg/µl). Both ethyl acetate and ethanol extracts had modest level of activity. Subsequent investigation of the most active extract (aqueous) against an *in vivo* model of *T. brucei brucei* infection showed a dose dependent trypanocidal activity. The extract reduced the rate of increase of parasitaemia and increased duration of survival of infected mice at a dose range of 50 - 400 mg/kg (Figure 1) compared to the vehicle control group in which there was a steep rise in the number of trypanosomes and 100 % mortality on the 5th day post - inoculation. The moderate DPPH – scavenging property of the aqueous extract of *G. lucidum* compared to rutin is shown in Table 3 while the antimicrobial activities are presented in Table 4. At a concentration of 250 mg/ml, the extract demonstrated broad spectrum activity against Gram positive and Gram-negative bacteria.

Table 1: Phytochemical Constituents of the Different Extracts of Fruiting Bodies of *G. lucidum*

	n-Hexane	Ethyl acetate	Ethanol	Water
Alkaloid	-	-	+++	-
Saponins	-	+	+	-
Tannin	-	-	-	+
Flavonoid	-	-	-	++
Carbohydrate	+	+	+	+
Anthraquinone	-	-	+	-
Glycoside	-	-	-	-+

- = Absent; + = Present

Table 2: *In vitro* Trypanocidal activity [IC_{50} (µg/µl)] of the Different Extracts of Fruiting bodies *G. lucidum*

	n-Hexane	Ethyl acetate	Ethanol	Water	Diminazine aceturate
G. lucidum	1500.92 ± 56.34	27.51 ± 3.41	83.12 ± 4.31	14.65 ± 2.54	0.98 ± 0.02

Values are mean ± S.E.M. N = 5

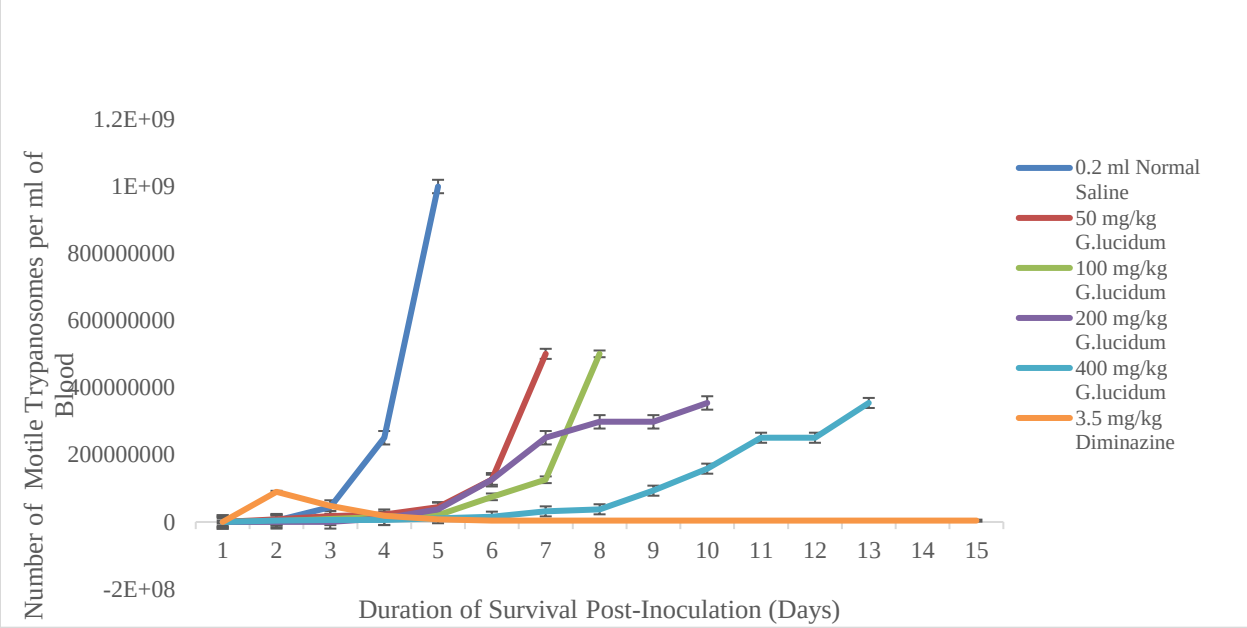

Figure 1: Effect of the aqueous extract of the fruiting bodies of *G. lucidum* on duration of survival and level of parasitemia in mice infected with *T. brucei brucei*

Table 3: DPPH scavenging activity of the aqueous extract of the fruiting bodies of G. *lucidum*

Test substance	IC_{50} (mg/ml)
Gallic Acid	$3.00x10^{-2} \pm 1.00x10^{-2}$
Rutin	$6.00x10^{-2} \pm 1.00x10^{-2}$
G. lucidum	$1.31x10^{2} \pm 0.29x10^{-1}$

Values are mean ± SEM N = 3

Table 4: Effect of the aqueous extract of the fruiting bodies of *G. lucidum* on bacterial and fungal zones of growth inhibition

Treatment	Concentration (mg/ml)	*P. aeruginosa*	*S. aureus*	*Bacillus subtilis*	*Candida albicans*
		Zone of inhibition (mm)			
Distilled water	0.2 ml	0	0	0	0
G. lucidum	62.5	10.83 ± 0.40*	0	0	0
G. lucidum	125.0	15.17 ± 0.79*	12.33 ± 0.33*	0	11.67 ± 0.56*
G. lucidum	250.0	22.83 ± 0.31*	18.67 ± 0.80*	19.00 ± 0.57*	18.00 ± 0.68*
Gentamicin	0.08	30.00 ± 1.29	34.50 ± 1.45	32.17 ± 0.83	-
Ketoconazole	0.02	-	-	-	18.83 ± 0.83

Values are mean ± S.E.M. N = 6 * $p < 0.05$

DISCUSSION

The result of the phytochemical screening of different extracts of the fruiting bodies of *G. lucidum* showed the presence of secondary metabolites such as carbohydrates, alkaloids, saponins, glycosides and flavonoids. These secondary metabolites occur in relatively small amounts compared to primary metabolites, yet they are of pharmacological and medicinal significance [26]. Alkaloids, carbohydrates and saponins are common constituents of the fruiting bodies of mushrooms and this agrees with results of published phytochemical studies of some

mushrooms [27,28]. The data presented also agree with the report of Okwulehie and Ogoke [29] who found a high alkaloid concentration in the fruiting bodies of some mushroom species. They observed that the caps had the highest amounts of alkaloids. The present study however, did not assay the relative distribution of alkaloids in the different parts of the mushroom.

The presence of phenolics (flavonoids, tannins) in the aqueous extract of *G. lucidum* agrees with the report by Abugri and Mcelhenney [30] who found a significant amount of flavonoids in both cultivated and wild mushrooms. Flavonoids are antioxidants and this property is a major health benefit of mushrooms; a relationship between increased intake of flavonoids and decreased incidence of adverse cardiovascular events has been reported [31]. Furthermore, antioxidants have a direct benefit in trypanosomiasis by mitigating free radical – induced lysis of red blood cells thereby improving the overall prognosis in infected animals [4,5]. The aqueous extract of *G. lucidum* used in this study was the terminal product of the sequential extraction process.

Table 2 shows the *in vitro* trypanocidal effects of the different extracts of *G. lucidum*, estimated as the inhibitory concentration 50 (IC_{50}). The IC_{50} in this study is the extract concentration that reduced the population of motile forms of the trypanosomes by half. It gives a measure of the potency and efficacy of the extracts, and offers an objective basis of comparing their activities. The degree of extract or drug activity is directly proportional to the population of exposed motile forms of the trypanosomes. The lower IC_{50} value of the aqueous extract suggests that it was the most active. The results further suggest the strong likelihood that the active principle(s) responsible for the observed trypanocidal activity may be hydrophilic in nature. This agrees with the findings of other investigators who reported that the aqueous extracts of plants parts demonstrated the greatest trypanocidal activity compared to other solvents [32,33,34]. Previous reports of the trypanocidal activity of the *G. lucidum* were not found, however, extracts of the fruiting bodies of *G. lucidum* have been reported to possess activity against bacteria and fungi, a property attributed to their content of phenolic compounds. The values obtained in the current *in vitro* trypanocidal studies are lower than those reported by Abedo et al [16] who reported values in the milligram per milliliter range. A number of factors may be responsible for this difference. Those authors used *T. Congolese* as against *T. brucei brucei*. It is possible therefore that the mushroom screened in this present study has greater potency against the organism used relative to the mushrooms investigated elsewhere.

The aqueous extract was found to be the most potent from the *in vitro* trypanocidal study and it was further evaluated for *in vivo* trypanocidal activity as well as anti – oxidant and anti – microbial actions. Our hypothesis was to screen extracts that demonstrated *in vitro* trypanocidal activity for *in vivo* trypanocidal, anti-oxidant and antimicrobial actions. Evaluating only the most active extract from the *in vitro* tests eliminated the need to subject experimental animals to extracts that were deemed ineffective. This is in line with the guidelines of 3R (replacement, reduction and refinement) which prescribe that use of experimental animals be only when absolutely necessary, and in as limited number as possible, without compromising the robustness and reproducibility of the experiment [35].

The effect of increasing doses of the extract in infected mice on the level of parasitaemia, expressed as the number of motile trypanosomes per ml of blood showed a slowing of the rate of increase of parasitaemia and a concomitant increase in the duration of survival of infected mice (Figure 1). This contrasted with the vehicle control group which showed a steep rise in parasitaemia and

death of all mice in the group by the 5th day. The expected outcome of an ideal trypanocidal agent is seen in the diminazine treated group. The reference drug diminazine aceturate (an aromatic diamidine) used in this experiment is an approved trypanocide. It can be observed that it killed the parasites and rendered the mice aparasitaemic by the 5th day post – inoculation. With undetectable parasites in the thin film, the animals survived for up to 30 days (not shown), in excess of both extract - treated and vehicle control groups. Although the precise mechanism of action diminazine is not known, it is thought to inhibit kinetoplast function by arresting cell cycle and the processing of DNA [36].

The reproduction of haemolymphatic trypanomastigotes is by an efficient binary fission process [37] which explains the rapid rise in parasitaemic levels in untreated infected mice. The ability of the extract to retard the buildup of parasitaemia indicates a trypanocidal effect. This is corroborated by the *in vitro* effect of the aqueous extract against *T. brucei brucei* parasites (Table 2). The strain of *T. brucei brucei* (Federe strain) used in the present study is highly virulent and the prolongation of the duration of survival of animals in the extract treated group is an important finding. In addition to retarding the rate of blood stream trypanosome multiplication, the observed increase in duration of survival may also be due to an indirect effect of the extract in enabling the animals cope with the burden of infection. Such coping mechanisms include immunomodulatory and anti – inflammatory activities of the extract (Unpublished data), properties now known to be possessed by diminazine [36]. The murine model of Human African Trypanosomiasis (HAT) is a robust and very useful experimental tool for screening of potential trypanocidal agents, especially in resource constrained settings. It makes possible direct estimation of parasitaemia from a peripheral blood smear and offers a quick evaluation of the effect of test substances [38]. This model was combined in this study with an *in vitro* model to provide evidence of the trypanocidal action of this aqueous extract.

This observed trypanocidal activity of the aqueous extract of the fruiting bodies of *G. lucidum* in *T. brucei brucei* infected mice has not previously been reported. The constituents of the aqueous extract were polyphenolic compounds (Table 1) and the trypanocidal activities of flavonoids have severally been reported [39,,40]. Though the mechanism of action of the extract was not determined, flavonoids have been reported to interfere with mitochondrial function in trypanosomes [41].

The antioxidant property of the aqueous extract of *G. lucidum* is beneficial in trypanosomiasis because the infection imposes an oxidative stress on infected animals due to the generation of free radicals. This oxidative stress is an important aetiologic factor in the haematological symptoms of infection and antioxidant therapy has been reported to mitigate trypanosomiasis – induced anaemia [42]. Antioxidant properties of natural products are attributable to their polyphenolic compounds such as flavonoids and tannins which stabilize free radicals by donating H^+ from the hydroxyl groups [43,44].

The aqueous extract of *G. lucidum*, within a concentration range of 62.5 – 250.0 mg/ml inhibited the growth of Gram positive and Gram-negative bacteria, and *Candida albicans* in the agar diffusion *in vitro* test (Table 4). Immune suppression is a well-documented complication of human African trypanosomiasis that makes the patient susceptible to infections by opportunistic pathogens [45,46]. The broad-spectrum action of the extract suggests its potential benefit as adjunctive therapy in the management of trypanosomiasis. Antimicrobial drugs act via a number of mechanisms, including inhibition of cell wall, protein and nucleic acid syntheses. Though the exact mechanism of action of the observed antimicrobial effect of the extract

was not investigated, the antimicrobial actions of flavonoids have been widely reported [47,48].

On the whole, the extract showed promise for further investigation and development into leads that could be used in the adjunctive management of HAT. These activities observed from the screening of extracts of a mushroom in this study indicates the benefit inherent in bioprospecting as a model of sourcing potentially useful leads from local biodiversity. Mushrooms can become important and substantial local sources of bioactive constituents due to their ease of cultivation from lignocellulosic wastes.

In conclusion, the aqueous extract of the fruiting body of *G. lucidum* demonstrated trypanocidal action and prolonged the duration of survival of mice infected with *T. brucei brucei.* It shows promise for the development of products useful in the management of human African trypanosomiasis.

Acknowledgement

The authors acknowledge the assistance of Late Mr. Paul Dalyop during the trypanocidal studies, the Nigerian Institute for Trypanosomiasis Research, Vom – Nigeria for kindly providing the parasite strain used in this study and The Africa Centre of Excellence for Phytomedicine Research and Development (ACEPRD), University of Jos, Jos for providing the solvents for this work.

REFERENCES

[1] Ibrahim MA, Mohammed A, Isah MB, Aliyu AB. Anti-trypanosomal activity of African medicinal plants: a review update. Journal of Ethnopharmacology 2014; 154 (1): 26–54.

[2] Wamwiri FN, Changasi RE. Tsetse Flies (Glossina) as vectors of human African trypanosomiasis : A review. BioMed Research International 2016. https://doi.org/10.1155/2016/6201350.

[3] Franco J, Simarro P, Diarra A, Jannin J. Epidemiology of human African trypanosomiasis. Clinical Epidemiology 2014; 6 (August): 257.

[4] Eze JI, Ajanachukwu N, Animoke PC, Onoja SO, Anosa GN, Eze UU. Immune response, anaemia and oxidative stress in Trypanosoma brucei brucei infected rats fed vitamin E supplemented diet. Anti-Infective Agents in Medicinal Chemistry. 2016 Volume 14, Issue 1, DOI: 10.2174/2211352514011603021221 53

[5] Mishra RR, Senapati SK, Sahoo SC, Das, MR, Sahoo G, Patra RC. Trypanosomiasis induced oxidative stress and haemato-biochemical alterations in cattle. Journal of Entomology and Zoology Studies 2107;5(6): 721 - 727

[6] Hotez PJ, Molyneux DH, Fenwick A, Kumaresan J, Sachs SE, Sachs JD, Savioli L. Control of neglected tropical diseases. The New Enlgand Journal of Medicine 2007; 357: 1018–27.

[7] Barrett MP, Isabel MV, Burchmore RJS, Kazibwe AJN, Matovu E. Drug resistance in human African trypanosomiasis. Future Microbiology 2011; 6 (9). 1037–47.

[8] Legros D, Ollivier G, Gastellu-Etchegorry M, Paquet C, Burri C, Jannin J, Büscher P. Treatment of human African trypanosomiasis—present situation and needs for research and development. The Lancet Infectious Diseases 2002; 2 (7): 437–40.

[9] Mwangi VI, Mumo RM, Nyachieo A, Onkoba N. Herbal medicine in the treatment of poverty associated parasitic diseases: A case of Sub-Saharan Africa. Journal of Herbal Medicine 2017; 10: 1–7.

[10] Peltzer K. Utilization and practice of traditional/complementary/alternative medicine (tm/cam) in South Africa. African Journal of Traditional, Complementary and Alternative Medicine 2009; 6 (2): 175–85.

[11] Freiburghaus F, Kaminsky R, Nkunya, MHH, Brun R. 'Evaluation of African Medicinal Plants for Their in Vitro Trypanocidal Activity. Journal of Ethnopharmacology 1996; 55 (1): 1–11.

[12] Ferreira CFR, Vaz JA, Vasconcelos MH, Martins A. Compounds from wild mushrooms with antitumor potential. Anti-Cancer Agents in Medicinal Chemistry 2010; 10 (5): 424–36.

[13] Ramesh C, Pattar MG. Antimicrobial properties, antioxidant activity and bioactive compounds from six wild edible mushrooms of Western Ghats of Karnataka, India. Pharmacognosy Research 2010; 2 (2): 107–12.

[14] Inchausti A, Yaluff G. Arias AR, Torres S, Ferreira ME, Nakayama H, … Fournet A. Leishmanicidal and trypanocidal activity of extracts and secondary metabolites basidiomycetes. Phytotherapy Research, 1997 1, 193-197.

[15] Cota BB, Rosa LH, Fagundes EMS, Martins-Filho

OA, Correa-Oliveira R, Romanha AJ, Rosa CA, Carlos-Zani CL . A potent trypanocidal component from the fungus lentinus *Strigosus* inhibits trypanothione reductase and modulates pbmc proliferation'. Mem Inst Oswaldo Cruz, Rio de Janeiro 2008;103 (3): 263–70.

[16] Abedo JA, Jonah OA, Abdullahi RS, Mazadu MR, Idris HY, Muhammed H, Shettima FT, et al. Evaluation of trypanosomal activity of *Tapinanthus globiferus* and *Gongronema latifolium* on *Trypanosoma congolense*. Bioscience Research 2013; 10 (1): 20–28

[17] Omar NAM, Abdullah N, Kuppusamy UR, Abdulla MA, Sabaratnam V. Nutritional composition, antioxidant activities, and antiulcer potential of *Lentinus squarrosulus* (mont.) mycelia extract. *Evidence-Based Complementary and Alternative Medicine* 2011. https://doi.org/10.1155/2011/539356.

[18] Dogan HH, Aydin S. Some biological activities of *Lactarius vellereus* (Fr.) Fr. In Turkey. Pakistan Journal of Biological Sciences 2013; 16 (21): 1279–86.

[19] Sanodiya BS, Thakur GS, Baghel RK, Prasad GBKS, Bisen PS. *Ganoderma lucidum*: A potent pharmacological macrofungus. Current Pharmaceutical Biotechnology 2009; 10 (8): 717–42.

[20] Veena SS, Pandey M. Medicinal mushrooms - a novel crop for horticultural diversification in India. *International Research Journal of Plant Science* 2012; 3 (1): 008-011.

[21] Sofowora A. Medicinal Plants and Traditional Medicine in Africa. (3rd ed.) 2008 Ibadan. Spectrum Books Limited.

[22] Brand-Williams W, Cuvelier ME, Berset C. Use of a Free Radical Method to Evaluate Antioxidant Activity. Food Sci. Technol. 1995; 28: 25–30.

[23] Usman A, Abdulrahman FI, Usman A. Qualitative phytochemical screening and in vitro antimicrobial effects of methanol stem bark extract of *Ficus thonningii* (Moraceae). African Journal of Traditional, Complementary and Alternative Medicines 2009; 6 (3): 289–95.

[24] Cheesbrough M. (2000), District Laboratory Practice in Tropical Countries Part 2, Cambridge Low-Price Editions, p 70

[25] Lamikrana A. (1999), Essential Microbiology for Students and Practitioners of Pharmacy, Medicine and Microbiology, Second Edition.

[26] Wink M. Modes of action of herbal medicines and plant secondary metabolites. Medicines 2015; 2 (3): 251–86.

[27] Ogbe AO, Ditse U, Echeonwu, Ajodoh K, Atawodi SE, Abdu PA. Potential of a wild medicinal mushroom, Ganoderma sp., as feed supplement in chicken diet: Effect on performance and health of pullets. International Journal of Poultry Science 2009; 8 (11): 1052–57.

[28] Bhanja SK, Rout D, Patra P, Sen IK, Nandan CK, Islam SS. Water-insoluble glucans from the edible fungus *Ramaria botrytis*. Bioactive Carbohydrates and Dietary Fibre 2014; 3 (2): 52–58

[29] Okwulehie IC, Ogoke, JA. Bioactive, nutritional and heavy metal constituents of some edible mushrooms found in Abia State of Nigeria. International Journal of Applied Microbiology and Biotechnology Research. 2013; 1: 7–15.

[30] Abugri DA, Mcelhenney WH. Extraction of total phenolic and flavonoids from edible wild and cultivated medicinal mushrooms as affected by different solvents. J . Nat . Prod . Plant Resource, 2013; 3 (3): 37–42.

[31] Verhoeyen ME, Bovy A, Collins G, Muir S, Robinson S, de Vos CHR, Colliver S. Increasing antioxidant levels in toamtoes through modification of the flavonoid biosynthetic pathway. Journal of Experimental Botany 2002; 53 (377): 2099–2106.

[32] Alli LA. Antitrypanosomal activity and haematological effects of aqueous extract of leaves of *Morinda lucida* on *Trypanosoma brucei brucei* infected rats. Asian Journal of Pharmaceutical and Health Sciences. 2011 1 (3)

[33] Ogunleye OO, Jatau ID, Natala AJ, Idehen CO, Salami O, Mamman SA. In vivo effect of the aqueous extract of *Adansonia digitata* (Linn) fruit pulp on *Trypanosoma brucei brucei* infection in Wistar rats. Sokoto Journal of Veterinary Sciences. 2019; 17 (4)

[34] Ayawa NG, Ramon-Yusuf SB, Wada YA, Oniye SJ, Shehu DM. Toxicity study and anti-trypanosomal activities of aqueous and methanol whole plant extracts of *Brillantaisia owariensis* on *Trypanosoma brucei-induced* infection in BALB/c mice. Clinical Phytoscience. 2021 Dec;7(1):1-1.

[35] Aske KC, Waugh CA. Expanding the 3R principles: More rigour and transparency in research using animals. EMBO reports. 2017, 18 (9); 1490 - 2

[36] Kuriakose S, Muleme H, Onyilagha C, Okeke E, Uzonna JE. Diminazine aceturate (Berenil) modulates LPS induced pro - inflammatory cytokine production by inhibiting phosporylation of MAPKs and STAT proteins. Innate Immunity 2014; 20 (7), 760 - 773

[37] Seed JR. Protozoa: Pathogenesis and Defenses, In, Baron S, editor. Medical Microbiology. 4th edition. Galveston (Texas, USA). University of Texas

Medical Branch at Galveston, 1996. Chapter 78

[38] Antoine-Moussiaux N, Magez S, Desmecht D. Contributions of experimental mouse models to the understanding of African trypanosomiasis. Trends in Parasitology 2008; 24 (9).

[39] Nwodo N, Okoye F, Lai D, Debbab A, Kaiser M, Brun R, Proksch P. Evaluation of the in vitro trypanocidal activity of methylated flavonoid constituents of *Vitex simplicifolia* leaves'. *BMC Complementary and Alternative Medicine* 2015; 15. https://doi.org/10.1186/s12906-015-0562-2.

[40] Nagagi YP, Silayo RS, Kweka EJ. Trypanocidal activity of ethanolic extracts of *Commiphora swynnertonii* burtt on *Trypanosoma congolense*. BMC Complemetary and Alternative Medicine 2016; 16: 195. https://doi.org/10.1186/s12906-016-1191-0.

[41] Marín C, Ramírez-Macías I, López-Céspedes A, Olmo F, Villegas N, Díaz JG, Rosales MJ, Gutiérrez-Sánchez R, Sánchez-Moreno M. In vitro and in vivo trypanocidal activity of flavonoids from *Delphinium staphisagria* against Chagas disease. Journal of Natural Products 2011; 74 (4): 744–50.

[42] Umar IA, Ogenyi E, Okodaso D, Kimeng E, Stancheva GI, Omage JJ, Isah S, Ibrahim MA. Amelioration of anaemia and organ damage by combined intraperitoneal administration of vitamins A and C to *Trypanosoma brucei brucei*-infected rats. African Journal of Biotechnology 2007; 6: 2083–86.

[43] Mohsin M, Negi PS, Ahmed Z. Determination of the antioxidant activity and polyphenol

contents of wild Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (W. Curt. Fr.) P. Karst.(Higher Basidiomycetes) from central Himalayan hills of India. International Journal of Medicinal Mushrooms, 2011; 13(6): 535-44

[44] Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. Journal of Nutritional Science 2016; 5 e47

[45] Vincendeau P, Bouteille B. Immunology and immunopathology of African trypanosomiasis. *Anais Da Academia Brasileira de Ciencias* 2006; 78 (4): 645–65.

[46] Baral TN. Immunobiology of African trypanosomes: Need of alternative interventions. Journal of Biomedicine and Biotechnology 2010;2010:389153. dio. 1155/2010/389153

[47] Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents 2005; 26 (5): 343–56.

[48] Dzoyem JP, Hamamoto H, Ngameni B, Ngadjui BT, Sekimizu K. Antimicrobial action mechanism of flavonoids from *Dorstenia* species. Drug Discoveries & Therapeutics 2013; 7 (2): 66–72.