In Vitro Trypanocidal Effect of the Extract and Isolated Compound of *Corymbia torelliana* Stem Barkagainst *Trypanosoma brucei brucei* (Federe Strain)

Abstract

Summary

African animal trypanosomosis (AAT) is a severe debilitating protozoan disease of livestock and some wild animal species. It is caused by the pathogenic trypanosome species, and transmitted by Tsetse flies. It affects about 60 million cattle in Africa, with about US$1.5 billion in annual losses. Eradication of the disease through chemotherapy and vaccine development has been hindered by parasite antigenic variation and its resistance to drugs. In this study, the crude ethanol stem bark extract of *Corymbia torelliana* was prepared by cold maceration in 70% Ethanol. It was further purified using the modified Kupchan partition method into N-hexane, Ethyl acetate, N-butanol and Aqueous pure fractions.A pure compound was isolated from the ethyl acetate purified fraction using the column and thin layer chromatographic techniques. The isolated compound was named Makoshi_Mak1. The *in vitro* antitrypanosomal effects of the crude extract, the purified fractions and the isolated compound were then tested against the blood stream forms of *Trypanosoma brucei brucei*(Federe strain). 0.2 ml of prepared trypanosome suspension containing about $2x10^4$ parasites was dispensed into each of seven clean test tubes using micropipettes. 0.2ml of prepared Ringer's solution was also added to stabilize the parasites. Five varying concentrations of the crude extract, the extract fractions and the isolated compound (Makoshi_Mak1) were added to test tubes 1-5. Test tube 6 and 7 served as the negative and positive controls respectively. Aliquots of the contents of each test tube were examined under the microscope at x400 magnification for parasites motility at different time interval for 2 hours. The crude extract was effective at the concentrations of 12 mg/ml, 1.2 mg/ml,0.12 mg/ml and 0.012 mg/ml. the N-hexane fraction had efficacy at 0.12mg/ml and 0.096mg/ml. The Eethyl acetate fractions were effective at 0.12mg/ml, 0.096 mg/ml, 0.048mg/ml and 0.024mg/ml, the N-butanol fraction had efficacy at 0.12mg/ml and 0.096mg/ml, while the Aqueous fraction was effective at 0.2mg/ml, 0.16mg/ml and 0.08mg/ml. Makoshi_Mak1 was effective *in vitro*against *T. brucei beucei* at all the tested concentrations of 0.219mg/ml, 0.109mg/ml, 0.055mg/ml and 0.027mg/ml. Efficacy of the extracts and isolated compound was determined by parasite mortality, and was seen to be concentration dependent. Dead parasites appeared to float freely on the fluid environment, while the lysed parasites were seen as floating debris.

Conclusion

The results of the study showed that both the crude extract, the purified extract and the pure isolated compound of *Corymbia torelliana* had *in vitro* activity against *Trypanosoma brucei brucei* in a concentration dependent manner. *Corymbia torelliana* can then be considered as a potential herbal antitrypanosomal drug candidate.

Introduction

African animal trypanosomosis (AAT), which is also called "Nagana," is a debilitating parasitic disease complex that affects different species of animals. The name "Nagana", is a Zulu terminology which means powerless or useless or "to be low or depressed in spirit" (Büscher *et al*., 2017). The disease is caused by flagellate protozoan parasites of the *genus Trypanosoma*and spread between animal hosts mainly by Tsetse flies (Glossina spp); which are the primary carriers and transmitters of nearly all the trypanosomes that cause AAT. They are mostly found along the coordinates of latitudes 15°N and 29°S, and are present throughout much of the African continent (Giordani et al., 2016).

The increasing incidences of African animal trypanosomosis resulting from the persistent and wide spread presence of biting flies has been one of Africa's main obstacles to sustained cattle production. (Medina & Mingala, 2016). In spite of the extensive Tsetse and trypanosomosis (T&T) control initiatives that have been put in place, Tsetse fly infestation has been spreading very rapidly within the regions of sub-Saharan Africa since the start of the 20th century and is hardly receding. The disease is becoming more and more difficult to control because of resistance developed by the parasites against most antitrypanosomal drugs (Meyer *et al*., 2016). Despite the fact that funding and interest in this field has been on the increase in the past decades, no new anti-trypanosomal drugs have been discovered or developed since the late 1980s. As a result, no significant progress has been made toward achieving this goal (Firdaus Abdullah Jesse et al., 2016)

In Africa, the prevention and treatment of AAT in domestic ruminants has mainly been hinged on three anti-trypanosomal drugs that have been used for many years, these include: (1) diminazene aceturate, (2) the Phenanthridine compounds (homidium and Isometamidium), (3) Quinapyramine (a quinoline pyrimidine). However, Quinapyramine is used only in camels and horses(Dagnachew et al., 2017).The prolonged and continuous utilization of these old compounds has been the cause of resistance development by the trypanosomes which no longer respond to these compounds. This resistance has been widely reported and is considered to be increasing. Antitrypanosomal drug resistance develop because trypanosomes are known to possess a glycoprotein coat known as variant surface glycoprotein (VSG) that is encoded by genes that enables it to go through the process of antigenic variation and shun the innate defense system of the infected animal host. This enables the parasites to develop resistance against most anti-trypanosomal drugs and also make vaccine development difficult (Wenzler *et al*., 2016)

The use of medicinal plants in the treatment of different diseases is known to have contributed significantly in the establishment of the modern pharmaceutical industry (Hale, 2013). This is because the knowledge of medicinal plants has had great impact in the development of modern pharmaceutical compounds used in drug research and development. Medicaments developed from ancient knowledge systems have been of tremendous significance in sustaining the wellbeing of societies through their use in the management of various types of illnesses. The ability to cure diseases by medicinal plants has been linked to the presence of biochemically active phytocompounds known as phytochemicals, which create functional changes within the body system to improve the body's physiological functions(Makoshi et al., 2013).These phytochemicals include primary metabolites such as fat, proteins and sugars, and secondary metabolites that include a variety of phytocompounds like cardiac glycosides, flavonoids, saponins and alkaloids (Santhi & Sengottuvel, 2015). These secondary group of compounds are associated with the medicinal functions that produce the healing effects observed when herbal medicines are used for treatment (Hoareau & DaSilva, 1999).

Materials and Methods

Plant Collection and Extraction

The stembarks of *Corymbia torelliana* were collected from the plants within the premises of the National Veterinary Research Institute Vom, Plateau State Nigeria. They were shade driedand pulverized into powder using a mortar and pestle; by repeated pounding and sieving. Plant extraction was carried out using cold macerationin 70% Ethanol(Deore *et al*., 2015). The extract's percentage yield of *Corymbia torelliana* was 17.4%.

Preparation of Ringers Solution

The Ringers solution used was prepared according to the modified method EZE et al (2007). The solution was prepared by weighing out 7.2 g of Sodium chloride (NaCl), 0.37g Potassium chloride (Kcl) and 0.17g Calcium chloride ($CaCl_2$). The salts were dissolved in 500ml of distilled water in a sterile container and topped up with another 500 ml of distilled water to make up the prepared solution's volume up to 1 liter. The solution's PH was then adjusted to 7.3. A filter paper with 0.22-m pore size was then used to filter the solution. After being autoclaved for sterilization, it was refrigerated at +4°C until it was needed. The solution was re-sterilized anytime it was needed.

Parasite Propagation and Determination of Parasitemia

The Federe strain of *Trypanosoma brucei brucei*was obtained from the National Institute of Tripanosomoiasis Research, Vom. The parasites were propagated in a male Wistar rat weighing 250g. At full parasitemia, Blood was collected from the donor rat into an Ethylene Diamine Tetra Acetic Acid (EDTA) bottle via the retro-orbital vein and diluted with 5% dextrose saline solution to produce a parasite suspension. Aliquots were taken from the parasites' suspension and examined at x400. The parasites Logarithm Equivalent Values (LEV) were calculated using the "Rapid Matching" method of Herbert and Lumsden (Ngutor *et al*., 2016).

Crude Extract Purification

Themodified Kupchan Partition method was used to prepare the purified extract fractions (Hossain et al., 2014), (Sharmin *et al*., (20 16).

Preparation of N-Hexane Fraction

50g of *Corymbia torelliana's*crude extract was weighed out into a clean container; 250ml of distilled water was then added and carefully stirred until the extract was completely dissolved. The dissolved extract was then poured into a 500ml separating funnel. 100ml of N-hexane was then added and properly mixed. The separating funnel was then vertically clamped to a retort stand and allowed to stand until a full separation of an upper N-hexane layer and a lower layer containing the residue in water were formed. The lower layer was then gently eluded through the tap of the separating funnel and collected into a beaker. The upper layer containing the N-hexane fraction was also collected using another beakerand filtered with No.1 Whatman's filter paper. The filtered fraction was then evaporated in an oven at 40^{o}C and stored for later use.

Preparation of Ethyl- Acetated Fraction

The residue obtained from the N-hexane extraction was poured into another 500ml separating funnel. 100 ml of Ethyl-Acetate was then added, gently mixed and mounted on a retort stand and allowed to stand until a full separation of an upper brownish layer containing the Ethyl-Acetated Fraction and a lower darker layer containing the residue in water were formed. The lower layer containing the residue in water was collected into a beaker. The upper layer of Ethyl- Acetated fraction was also collected into another beaker and filtered with Whatman's filter paper. The filtered ethyl- acetated fraction was then dried in anoven at and stored for later use.

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Preparation of N-Butanol Fraction

The residue from the Ethyl- Acetated Fraction was transferred into another separating funnel. 100 ml of N-Butanol was then added and properly mixed. The mixture was then mounted on a retort stand and allowed to stand until an upper layer of N-Butanol fraction and a lower layer of the residue were formed. The lower residue layer was collected into a beaker and kept separately. The upper N-Butanol fraction layer was also collected and filtered with Whatman's filter paper. The collected fraction was evaporated and dried in an oven at 40^{o}C.

Preparation the Aqueous Fraction.

The aqueous fraction was prepared by filtering the residue in water using a 0.22-µm pore size Whatman's filter paper.It was then dried in the oven at the same temperature. Each of the above procedures were repeated thrice for proper removal of the solvents.

Compound Isolation

The compound isolation from the ethyl acetate purified fractionwas carried out using the column and thin layer chromatographic techniques described by (Kabir et al., 2021). The glass column used measured 50 cm3 long and 3.5cm wide. Silica gel of 60-120 Mesh (Qualikems) was used as the stationary phase for the column. N-hexane and Ethyl acetate were combined at ratio 2: 8 for the mobile phase for the Column. The mobile phase for the TLC developing tank was at 3:7. A total of 867 aliquots were collected into 25ml bottles. TLC checks were carried out on each collected sample. Samples with similar retention factor values (RF) were pooled together into 12 major sub fractions. Sub fractions 1-6 showed the same TLC profiles and were pulled together as an isolate. Further TLC checks were made to confirm the RF values. The isolated compound was named Makoshi_Mak1. It was further dried to crystals and used for the *in vitro* antitrypanosomal study

In-Vitro Antitrypanosomal Assay of the Crude Extract

5 test tube racks were arranged on four different work benches containing seven clean test tubes labelled 1-7. 0.2ml of the trypanosome suspension containing about 2x10^{4} parasites was dispensed into each of seven clean test tubes using a micropipette. 0.2ml of the prepared Ringer's solution was also added to all the test tubes to stabilize the parasites. 0.2ml of each of the 5 different concentrations (20mg/ml, 2mg/ml, 0.2mg/ml, 0.02mg/ml and 0.002mg/ml) of each of the crude extracts were then added to test tubes 1 to 5, making the final extract concentrations in test tubes 1-5 to be 12mg/ml, 1.2mg/ml, 0.12mg/ml, 0.012mg/ml and 0.0012mg/ml respectively. 0.2ml of physiological normal saline was added to test tube 6

which served as the negative controls and 0.2ml of reconstituted Diminazen aceturate (Berenil®) was added to test tubes 7, which served as the positive controls. The mixtures were gently mixed in the test tubes and kept in the rags. Aliquots of the contents of each test tube were examined under the microscope for parasites motility at x400 magnification. The time of first examination was noted as 0minute. The test tubes and their contents were then incubated at 37^{o}C immediately. The second readings were taken after 5minutes, but subsequent readings were taken after every 15minutes. The test tubes and their contents were re-incubated after every reading was taken. The examinations lasted for 2hours; after which the experiments were terminated. The observations made after every reading were recorded

In-Vitro Antitrypanosomal Evaluation of the Purified Fractions of *Corymbia torelliana*

5 stock concentrations of the purified fractions were prepared at 2mg/ml, 0.16mg/ml, 0.02mg/ml, 0.04mg/ml and 0.08mg/ml. four test tube racks containing 7 test tubes labelled 1-7 were arranged on work benches. 0.2ml of the trypanosome suspension containing about 2x10^{4} parasites was dispensed into each of seven clean test tubes using a micropipette. 0.2ml of Ringer's solution was added to all the test tubes to stabilize the parasites. 0.2ml of each varying concentrations of the purified fractions was added to test tubes 1 to 5 making a final extract concentration in test tubes 1-5 to 0.12mg/ml, 0.096mg/ml, 0.048mg/ml, 0.024mg/ml and 0.012mg/ml respectively. 0.2ml of physiological normal saline was added to test tube 6 (negative control), and 0.2ml of 7% Diminazen aceturate (Berenil®) was added to test tube 7 (positive control). The mixture was gently mixed in the test tubes and kept in the rags. Aliquots of the test tube contents were taken and examined under the microscope for parasites motility at x400 magnification. The time of first examination was noted as 0 minute. The test tubes and their contents were then incubated at 37^{o}C immediately. The second readings were taken after 5 minutes, but subsequent readings were taken after every 15 minutes. The test tubes and their contents were re-incubated after every reading was taken. The examination lasted for 2 hours after which the experiment was terminated.

In vitro Antitrypanosomal Effect of the Isolated Compound

The antitrypanosomal effect of the isolated compound was tested *in vitro* against *Trypanosoma brucei brucei.* Four stock concentrations of the isolated compound were prepared at 0.35mg/ml, 0.175mg/ml, 0.0875 mg/ml and 0.0438mg/ml. 0.2ml of *Trypanosoma brucei brucei* suspension containing about 2x10^{4} parasites was prepared and dispensed into each of six test tubes using a micropipette. 0.2ml of Ringer's solution was added to all the test tubes to stabilize the parasites. 0.2ml of each of the four stock concentrations of the isolated compound was added to test tubes 1 to 4, bringing the final concentration of the compound in test tubes 1-4 to 0.219mg/ml, 0.109mg/ml, 0.055mg/ml and 0.027mg/ml respectively. 0.2ml of physiological normal saline was added to test tube 5 (negative control), and 0.2ml of 7% Diminazen aceturate (Berenil®) was added to test tube 6 (positive control). The preparation was gently mixed in the test tubes, and few drops were taken from each test tube and examined under the microscope for parasites motility at x400 magnification. The time of first examination was noted as 0 minute. The test tubes and their contents were then incubated at 37^{o}C immediately. The second readings were taken after 5 minutes, but subsequent readings were taken after every 15 minutes. The test tubes and their contents were re-incubated after every reading was taken. The examination lasted for 2 hours after which the experiment was terminate

RESULTS

General Key: **++++** = *Parasites are very active,* **+++** = *Parasites are active but not very active,* **++** = *parasites are sluggish,* **+** = *parasites are very sluggish,* **-** = *No Parasites seen,* ***APD*** = *All Parasites dead. Dead parasites were seen floating freely and inactive*

Table 1. The *In vitro* Anti-trypanosomal Effect of the Crude Extract of *Corymbia torelliana* on *Trypanosoma brucei brucei*(Federe strain)

Conc. of E.T. extract	Observations Made Within Time Intervals (minutes)									
	0 Min	5 Min	15 Min	30 Min	45 Min	60 Min	75 Min	90 Min	105 Min	120 Min
12 mg/ml	+++	-	-	-	-	-	-	-	-	-
1.2 mg/ml	+++	-	-	-	-	-	-	-	-	-
0.12 mg/ml	++++	++	-	-	-	-	-	-	-	-
0.012 mg/ml	++++	++	-	-	-	-	-	-	-	-
0.0012mg/ml	++++	++++	++	++	++	+	+	+	+	+
-Ve Control	++++	++++	++++	++++	++++	++++	+++	+++	+++	+++
+Ve Control	++	-	-	-	-	-	-	-	-	-

At 12mg/ml, 1.2mg/ml and 0.12mg/ml, all parasites died within 15 minutes. At 0.012mg/ml, all the parasites died within 45minute. However, at 0.0012mg/ml, all parasitessurvived, most of which moved only very slowly. All parasites in the negative control group were active throughout the time of the experiment. All parasites in the group administered Diminazen aceturate (Berenil®) died within 15 minutes.

Table 2. The *In-Vitro*Trypanocidal Effect of N-Hexane Fraction of *Corymbia torelliana* on Trypanosoma *brucei brucei*

Conc. of NHF	Observations Made Within Time Intervals (minutes)									
	0 Min.	5 Min	15 Min	30 Min	45 Min	60 Min.	75 Min	90 Min	105 Min	120 Min
0.12 mg/ml	++++	++++	++++	++	+ 25 % Dead	+ 50 % Dead	+ 80 % Dead	+ 95 % Dead	APD	APD
0.096 mg/ml	++++	++++	++++	++	10 % Dead	30% Dead	60 % Dead	90 % Dead	APD	APD
0.048 mg/ml	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
0.024 mg/ml	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
0.012 mg/ml	++++	++++	++++	++++	++	++++	++++	++++	++++	++++
-Ve Control	++++	++++	++++	++++	++++	++++	++++	+++	+++	++
+Ve Control	++	-	-	-	-	-	-	-	-	-

At 0.12mg/ml and 0.096 mg/ml, the parasites activities slowed down, and all died within 30-45 minutes. At 0.048mg/ml, 0.024mg/ml, and 0.012mg/ml, the parasites were seen to be active throughout the 2 hours period of observation. Parasites in the negative control group were also very active throughout the examination period. However, the parasites in the positive control group treated with Diminazen aceturate all died within 15 minutes.

Table 3. The In-Vitro Anti-trypanosomal Effect of Ethyl-acetate Fraction of *Corymbia torelliana* on *Trypanosoma brucei brucei*

Conc. of NBF	Observations Made Within Time Intervals (minutes)									
	0 Min.	5 Min	15 Min	30 Min	45 Min	60 Min.	75 Min	90 Min	105 Min	120 Min
0.12 mg/ml	++++	++++	++++	++	+ 25 % Dead	+ 50 % Dead	+ 80 % Dead	+ 95 % Dead	APD	APD
0.096 mg/ml	++++	++++	++++	++	10 % Dead	30% Dead	60 % Dead	90 % Dead	APD	APD
0.048 mg/ml	++++	++++	++++	++++	+ 20% Dead	+ 35 % dead	+ 50 % dead	+ 70 % dead	90% dead	APD
0.024 mg/ml	++++	++++	++++	++++	++++	++++	++ 60% dead	++ 70% dead	+ 90 % dead	APD
0.012 mg/ml	++++	++++	++++	++++	++	++++	++ 35 % dead	+ 40 % dead	+ 70% dead	+ 80% dead
-Ve Control	++++	++++	++++	++++	++++	++++	++++	+++	+++	++
+Ve Control	++	-	-	-	-	-	-	-	-	-

At 0.12mg/ml and 0.096 mg/ml the parasite motility was initially very slow in the first 30minutes. Dead parasites were seen after 45 minutes. All parasites died within 90 minutes. At 0.08mg/ml 0.04mg/ml, parasites motility was high from the beginning, but slowed down progressively. All parasites died within 2 hours. At 0.02 mg/ml, both dead and very sluggishly moving parasites were seen throughout the 2 hours study period. Parasites in the negative control group were very active throughout the study period, but those in the positive control group all died within 15 minutes.

Table 4. The *In vitro* Effect of N-butanol Fraction of *Corymbia torelliana* on *Trypanosoma Brucei Brucei* (Federe Strain)

Conc. Of Ethyl - Acetate Fraction	Observations Made Within Time Intervals (minutes)									
	0 Min.	5 Min	15 Min	30 Min	45 Min	60 Min.	75 Min	90 Min	105 Min	120 Min
0.2 mg/ml	++++	+++ +	+++ +	+++ +	++++	++ 20% dead	+ 75 % dead	+ 80 % dead	+ 95 % dead	APD
0.16 mg/ml	++++	+++ +	+++ +	+++ +	+++	++	+ 20% dead	+ 50 % dead	+ 90 % dead	APD
0.08 mg/ml	++++	+++ +	+++ +	+++ +	++++	++++	+++	++	+	+
0.04 mg/ml	++++	+++ +	+++ +	+++ +	++++	++++	+++	++	+	+
0.02 mg/ml	++++	+++ +	+++ +	+++ +	+++	+++	++	++	++	++
- Ve Control	++++	+++ +	+++ +	+++ +	++++	+++	+++	+++	++	++
+Ve Control	++	-	-	-	-	-	-	-	-	-

At 0.12mg/ml and 0.096mg/ml, the parasites were initially very active, but their motility started to slow down. Within 1 to 2 hours, all parasites in this group were dead. At the concentration of 0.048, 0.024 and 0.012mg/ml, the parasites' motility progressively decreased with time. Few dead parasites were seen even at the end of the 2 hours period of observation. Parasites in the negative control group were very active throughout the study period, but those in the positive control group all died within 15 minutes.

Table 5. The *In vitro* Effect of The Aqueous Fraction of *Corymbia torelliana* on *Trypanosoma Brucei Brucei*(Federe Strain)

Conc. Of Water Fraction	Observations Made Within Time Intervals (minutes)									
	0 Min.	5 Min	15 Min	30 Min	45 Min	60 Min.	75 Min	90 Min	105 Min	120 Min
0.2 mg/ml	+++ +	++++	+++	++++	++	++ 20% dead	+ 75% dead	+ 80% dead	+ 95% dead	APD
0.16 mg/ml	+++ +	++++	++++	+++	++	++ 20% dead	+ 50% dead	+ 70% dead	+ 90% dead	APD

0.08 mg/ml	+++ +	++++	++++	++++	++++	++ 20% dead	+++ 40% dead	++ 75% dead	+ 90% dead	APD
0.04 mg/ml	+++ +	++++	+++	+++	+++	++ 20 % dead	++ 20 % dead	++ 20 % dead	+ 20 % dead	+ 20% dead
0.02 mg/ml	+++ +	++++	++++	+++	+++	+++	++ 10% dead	++ 10% dead	+ 20% dead	+ 20% dead
-Ve Cont.	+++ +	++++	++++	++++	++++	+++	+++	+++	++	++
+Ve Cont.	++	-	-	-	-	-	-	-	-	-

At 0.2mg/ml, 0.16mg/ml and 0.08mg/ml, parasites in these group appeared were very active in the first 45 minutes, their activities then slowed down as the extract took effect. All parasites in these groups died within 2 hours. At 0.04mg/kg and 0.002mg/kg, both dead and slow-moving parasites were seen throughout the 2 hours. Parasites in the negative control group were very active throughout the study period, but those in the positive control group all died within 15 minutes.

Table 6. The *In-Vitro* Anti-trypanosomal Effect of the Isolated Compound on *Trypanosoma brucei brucei*

Conc. of NBF	**Observations Made Within Time Intervals (minutes)**									
	0 Min.	**5 Min**	**15 Min**	**30 Min**	**45 Min**	**60 Min.**	**75 Min**	**90 Min**	**105 Min**	**120 Min**
0.219 mg/ml	++++	++++	++50% Dead	++ 90% dead	D	D	D	D	D	D
0.109 mg/ml	++++	+++	+++	++	50 % Dead	99% Dead	D	D	D	D
0.055 mg/ml	++++	++++	++++	++++	+	+ 60 % dead	+ 70 % dead	D	D	D
0.027 mg/ml	++++	++++	++++	++++	+++	++	+	80% dead	D	D
-Ve Control	++++	++++	++++	++++	++++	++++	++++	+++	+++	++
+Ve Control	++	-	-	-	-	-	-	-	-	-

At the concentration of 0.219mg/ml (group), the parasitemotility was initially very fast, but later slowed down. All the parasite died within 45 minutes. At 0.109mg/ml, the parasites died within 75 minutes. At 0.055 mg/ml, all the parasites died within 90 minutes. At 0.027mg/ml all parasites died within 05 minutes. The parasites in the negative control group were very

active throughout the study period, but those in the positive control group all died within 5 minutes.

DISCUSSION

In this study, the decrease in the parasite motility or the complete cessation of the parasites' activity was considered as a measure of the antitrypanosomal activity of the crude extract,the purified extract fractions and the isolated compound. The study revealed that the stem bark extracts of *Corymbia torelliana* had *in vitro* antitrypanosomal effects either as crude extracts purified fractions or the isolated compound against the blood stream forms of *Trypanosoma brucei brucei* (Federe strain), and the activity was concentration dependent. These findings were in agreement with the findings ofOgbole *et al.*, (2016), who also discovered that the crude leaf extract of *Corymbia torelliana* had *in vitro* activity against *Trypanosoma brucei crucei*.

The high antitrypanosomal effect of the test compounds observed was indicative of the high potency of the stem bark extract of *Corymbia torelliana*against *Trypanosoma brucei crucei*. Although the parasites were initially very active when introduced to the varying concentrations of the extract at the beginning of the experiments, their activities began to slow down as the phytocompounds within the extracts began to take effect.

Although the trypanocidal effect of the test compounds was seen to be concentration dependent, most of the tested concentrations produced trypanocidal effects in all the test samples, especially in the crude extract, the Ethyl acetate fraction and the isolated compound (Makoshi_Mak1). Higherconcentrations of these test samples produced faster trypanocidal effects. This shows that higher concentrations of plant compounds may contain more phytocompounds which in turn produces faster biochemical or antimicrobial effects. On the other hand, very low concentrations that could not cause parasite mortality could be said to contain vey low amounts of phytocompounds that were too weak to elicit any antitrypanosomal effects.

During the study, all parasites in the negative control groups which had no plant extracts but only physiological solutionsdid not die, but were active throughout the time of the experiment. This shows that the death of the parasites in the test groups was as result of the phytocompounds present in the extracts and the compound isolated from the stem bark of *Corymbia torelliana.*

The extracts and isolated compound tested in this study produced trypanocidal effectsthat were comparative to those produced by Diminazen aceturate (Berenil®) in the positive control groups, in which all the parasites died within fifteen minutes post exposure. The antitrypanosomal effect of the crude extract at 12mg/ml, 1.2mg/ml and 0.12mg/ml did not just produce mortality of the parasites but also caused lyses of the death parasites. The dead parasites appeared to float freely on the fluid environment, while the lysed parasites were seen as floating debris. The extract fractions were also seen to produce dose dependent trypanocidal effects. The Ethyl acetate fraction showed better antitrypanosomal effect, producing parasites deaths at almost all the tested concentrations of 0.12mg/ml, 0.096 mg/kg, 0.048 mg/kg, and 0.024 mg/kg.

The trypanocidal effect of the extract of *Corymbia torelliana* shown in this study could be attributed to the presence of secondary phytochemicals in the extracts; whichare known to have antimicrobial properties and are responsible for the therapeutic effects of most medicinal plants(Adeniyi & Ayepola, 2008). These findings are also in agreement with the

findings of Bawm *et al*, (2010) and Ogbole *et al*, (2016) who carried out the in-vitro antitrypanosomal evaluations of the leave extracts of *Corymbia torelliana*and showed that they possessed in-vitro trypanocidal effects against *Trypanosoma brucei brucei*.

Conclusion

This study revealed that the crude extract, the purified extract fractions and the isolated compound (Makoshi_Mak1) obtained from the stem bark of *Corymbia torelliana* have very good *in vitro* antitrypanosomal activities, and could serve as potential drug candidates for the development of plant based trypanocidal medicaments.

REFERENCES

Adeniyi, B. A., & Ayepola, O. O. (2008). The Phytochemical Screening and Antimicrobial Activity of Leaf Extracts of Eucalyptus camaldulensis and Eucalyptus torelliana (Myrtaceae). In *Research Journal of Medicinal Plant* (Vol. 2, Issue 1, pp. 34–38). https://doi.org/10.3923/rjmp.2008.34.38

Bawm, S., Tiwananthagorn, S., Lin, K. S., Hirota, J., Irie, T., Htun, L. L., Maw, N. N., Myaing, T. T., Phay, N., Miyazaki, S., Sakurai, T., Oku, Y., Matsuura, H., & Katakura, K. (2010). Evaluation of Myanmar medicinal plant extracts for antitrypanosomal and cytotoxic activities. *The Journal of Veterinary Medical Science / the Japanese Society of Veterinary Science*, *72*(4), 525–528. https://doi.org/10.1292/jvms.09-0508

Büscher, P., Cecchi, G., Jamonneau, V., & Priotto, G. (2017). *Human African trypanosomiasis*. *390*, 2397–2409. https://doi.org/10.1016/S0140-6736(17)31510-6

Dagnachew, S., Tsegaye, B., Awukew, A., Tilahun, M., Ashenafi, H., Rowan, T., Abebe, G., Barry, D. J., Terefe, G., & Goddeeris, B. M. (2017). Prevalence of bovine trypanosomosis and assessment of trypanocidal drug resistance in tsetse infested and non-tsetse infested areas of Northwest Ethiopia. *Parasite Epidemiology and Control*, *2*(2), 40–49. https://doi.org/10.1016/j.parepi.2017.02.002

Deore, S. L., Baviskar, B. A., & Rangari, A. S. (2015). Rapid and high yield Extraction method for Saponins from Safed musli. *Pharmacognosy Journal*, *7*(4), 210–214. https://doi.org/10.5530/pj.2015.4.1

EZE, J. I., ANOSA, G. N.* and OZOTA, C. A. (2007). Invitro and in Vivo Trypanocidal Activitiy of Combretumracemosum Leaves. *Nigerian Veterinary Journal*, *32*(4), 342–348.

Firdaus Abdullah Jesse, F., Abubakar Sadiq, M., Umar Hambali, I., Lim Teik Chung, E., Lau Pick Ping, F., Wahid Haron, A., Aziz Saharee, A., Jefri Norsidin, M., Athliamai Bitrus, A., Abba, Y., Azmi Mohd Lila, M., Faez Firdaus Abdullah Jesse, C., & Wahid Haron, A. (2016). A clinical case of bovine trypanosomosis in an endemic farm in Malaysia http://bdvets.org/javar. *Vet. Anim. Res. Journal of Advanced Veterinary and Animal Research Adv. Vet. Anim. Res*, *3*(33), 286–291. https://doi.org/10.5455/javar.2016.c164

Giordani, F., Morrison, L. J., Rowan, T. I. M. G., Koning, H. P. D. E., & Barrett, M. P. (2016). *The animal trypanosomiases and their chemotherapy : a review*. 1862–1889. https://doi.org/10.1017/S0031182016001268

Hale, A. (2013). Microscope. *Journal of Chemical Information and Modeling*, *53*, 1689–1699. https://doi.org/10.1017/CBO9781107415324.004

Hoareau, L., & DaSilva, E. J. (1999). Medicinal plants: A re-emerging health aid. *Electronic Journal of Biotechnology*, *2*(2), 56–70. https://doi.org/10.2225/vol2-issue2-fulltext-2

Hossain, M. A., Al-Hdhrami, S. S., Weli, A. M., Al-Riyami, Q., & Al-Sabahi, J. N. (2014). Isolation, fractionation and identification of chemical constituents from the leaves crude extracts of Mentha piperita L grown in Sultanate of Oman. *Asian Pacific Journal of Tropical Biomedicine*, *4*(Suppl 1), S368–S372. https://doi.org/10.12980/APJTB.4.2014C1051

Kabir, N., Umar, I. A., Dama, H. A., James, D. B., & Inuwa, H. M. (2021). Isolation and Structural Elucidation of Novel Antidiabetic Compounds from Leaves of Momordica balsamina Linn and Leptadenia hastata (Pers) Decne. *Iranian Journal of Pharmaceutical Research*, *20*(2), 390–402. https://doi.org/10.22037/ijpr.2020.113632.14440

Makoshi, M. S., Adanyeguh, I. M., & I, L. I. N. (2013). Hepatoprotective effect of Phyllanthus niruri aqueous extract in acetaminophen sub-acutec exposure rabbits. *Journal of Veterinary Medicine and Animal Health*, *5*(January), 8–15. https://doi.org/10.5897/JVMAH12.004

Medina, N. P., & Mingala, C. N. (2016). Transporter protein and drug resistance of Trypanosoma. *Annals of Parasitology*, *62*(1), 11–15. https://doi.org/10.17420/ap6201.26

Meyer, A., Holt, H. R., Selby, R., & Guitian, J. (2016). Past and Ongoing Tsetse and Animal Trypanosomiasis Control Operations in Five African Countries: A Systematic Review. *PLoS Neglected Tropical Diseases*, *10*(12), 1–29. https://doi.org/10.1371/journal.pntd.0005247

Ngutor, K. S., Idris, L. A., & Oluseyi Oluyinka, O. (2016). Silent Human Trypanosoma brucei gambiense Infections around the Old Gboko Sleeping Sickness Focus in Nigeria. *Journal of Parasitology Research*, *2016*. https://doi.org/10.1155/2016/2656121

Ogbole, E., Dashak, D. A., Nvau, J. B., Daben, M. R., Abongaby, G., Obaloto, O. B., & Oladipo, O. O. (2016). *Phytochemical screening and in vitro evaluation of the antitrypanosomal action of the methanolic leaf extract of Corymbia torelliana*. *3*(1).

Santhi, K., & Sengottuvel, R. (2015). *Qualitative and Quantitative Phytochemical Analysis of* (Vol. 5, Issue April, pp. 633–640). https://doi.org/10.13040/IJPSR.0975-8232.6(1).393-98

Sharmin, S., Islam, Z., & Jabber, A. (2016). *Bioscience and Bioengineering Communications Isolation and identification of medicinal compounds from Kalanchoe pinnata of Crassulaceae family by 1 H NMR*. *2*(2), 123–129.

Wenzler, T., Burkard, G. S., Schmidt, R. S., Bergner, A., Roditi, I., & Brun, R. (2016). A new approach to chemotherapy: Drug-induced differentiation kills African trypanosomes. *Scientific Reports*, *6*(February), 1–10. https://doi.org/10.1038/srep22451

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