

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

### Abstract

#### Summary

African animal trypanosomiasis (AAT) is a severe debilitating protozoan disease of livestock and some wild animal species. It is caused by [parasites in](#) the genus *Trypanosoma*, 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 *in vitro* antitrypanosomal effects of the [aqueous, ethanolic, methanolic](#) crude extract, the purified extracts and a pure compound isolated from the stem bark of *Corymbia torelliana* were tested *in vitro* against *Trypanosoma brucei* (Federe strain). All the tested samples showed efficacy against the parasite at varying concentrations in a concentration dependent manner. The crude extract was effective at the concentrations of 12mg/ml, 1.2mg/ml, 0.12mg/ml and 0.012mg/ml. The purified extract fractions were also effective at varying concentrations in the following manner: the N-hexane fraction had efficacy at 0.12mg/ml and 0.096mg/ml. The Ethyl 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. The isolated compound ([which compound?](#)) ([Makoshi\\_Mak1](#)) was effective *in vitro* against *T. brucei* at all the tested concentrations of 0.219mg/ml, 0.109mg/ml, 0.055mg/ml and 0.027mg/ml.

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#### 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* in a concentration dependent manner. *Corymbia torelliana* can then be considered as a potential herbal antitrypanosomal drug candidate.

#### Introduction

African animal trypanosomiasis (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 (Wamwiri, 2016).

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The increasing incidences of African animal trypanosomiasis 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

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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 (Brun *et al.*, 2010).

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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 (Tsegaye *et al.*, 2015). 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).

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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 phytochemicals known as phytochemicals, which create functional changes within the body system to improve the body's physiological functions (Evans, 2009). These phytochemicals include primary metabolites such as fat, proteins and sugars, and secondary metabolites that include a variety of phytochemicals 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).

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## 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?, oven?dried and pulverized into powder using a mortar and pestle. Plant extraction was carried out using cold maceration in 70% Ethanol (Deore *et al.*, 2015). The extract's percentage yield of *Corymbia torelliana* was 17.4%.

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### Preparation of Ringers Solution

The Ringers solution used was prepared according to the modified method of [Petana \(1964\)](#). 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<sub>2</sub>). 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-μ 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.

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### Parasite Propagation and Determination of Parasitemia

The Federe strain of *Trypanosomabrucei* was obtained from the National Institute of Tripanosomiasis 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](#)).

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### Crude Extract Purification

The modified Kupchan Partition method was used to prepare the purified extract fractions ([Hossain et al., 2014](#)), ([Sharmin et al., 2016](#)).

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### Preparation of N-Hexane Fraction

50g of *Corymbiatarelliana*'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 beaker. It was then filtered using a 0.22μm pore size filter paper and evaporated in an oven at 40°C. The extract fraction was then collected and stored for later use.**

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Which component was subjected for filtering using a 0.22μm pore size filter paper and evaporation in the oven? Was it the upper or lower layer?

### 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 using a 0.22μm pore size filter paper. The collected Ethyl- Acetated Fraction was then dried in an oven at 40°C.

### 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 the 0.22-µm porosity Whatman's filter paper. **The collected fraction was evaporated and dried in an oven at 40°C. (which one? The lower? The Upper)**

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### 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 **twice or thrice** for proper removal of the solvents.

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### Compound Isolation

**The compound isolation from** was carried out using the column and thin layer chromatographic techniques described by **Kabir et al., (2021)**. The glass column used measured 50 cm<sup>3</sup> 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 further dried to crystals and used for the *in vitro* antitrypanosomal study

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### 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  $2 \times 10^4$  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 acetate (Berenil<sup>®</sup>) 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 racks. 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°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 *Corymbiaturelliana***

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  $2 \times 10^4$  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<sup>®</sup>) was added to test tube 7 (positive control). The mixture was gently mixed in the test tubes and kept in the racks. 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°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 *Trypanosomabruceibrucei*. Four stock concentrations of the isolated compound were prepared at 0.35mg/ml, 0.175mg/ml, 0.0875g/ml and 0.0438mg/ml. 0.2ml of *Trypanosomabruceibrucei* suspension containing about  $2 \times 10^4$  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<sup>®</sup>) 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°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 *Corymbiaturelliana* on *Trypanosomabruceibrucei*(Federe strain)**

| Conc. of<br>E.T. extract | Observations Made Within Time Intervals (minutes) |       |           |           |           |           |           |           |            |            |
|--------------------------|---|-------|-----------|-----------|-----------|-----------|-----------|-----------|------------|------------|
|                          | 0 Min   | 5 Min | 15<br>Min | 30<br>Min | 45<br>Min | 60<br>Min | 75<br>Min | 90<br>Min | 105<br>Min | 120<br>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<sup>®</sup>) died within 15 minutes.

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

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

| 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   | ++++  | ++++  | ++++   | ++     | +         | +         | +         | +         | APD       | APD      |
|              |   |       |        |        | 25 % Dead | 50 % Dead | 80 % Dead | 95 % Dead |           |          |
| 0.096 mg/ml  | ++++  | ++++  | ++++   | ++     | 10 % Dead | 30% Dead  | 60 % Dead | 90 % Dead | APD       | APD      |
| 0.048 mg/ml  | ++++  | ++++  | ++++   | ++++   | +         | +         | +         | +         | 90% dead  | APD      |
|              |   |       |        |        | 20% Dead  | 35 % dead | 50 % dead | 70 % dead |           |          |
| 0.024 mg/ml  | ++++  | ++++  | ++++   | ++++   | ++++      | ++++      | ++        | ++        | +         | APD      |
|              |   |       |        |        |           |           | 60% dead  | 70% dead  | 90 % dead |          |
| 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                         | ++++  | +++<br>+ | +++<br>+ | +++<br>+ | ++++   | ++<br>20%<br>dead | +<br>75 %<br>dead | +<br>80 %<br>dead | +<br>95 %<br>dead | APD     |
| 0.16 mg/ml                        | ++++  | +++<br>+ | +++<br>+ | +++<br>+ | +++    | ++                | +<br>20%<br>dead  | +<br>50 %<br>dead | +<br>90 %<br>dead | APD     |
| 0.08 mg/ml                        | ++++  | +++<br>+ | +++<br>+ | +++<br>+ | ++++   | ++++              | +++               | ++                | +                 | +       |
| 0.04 mg/ml                        | ++++  | +++<br>+ | +++<br>+ | +++<br>+ | ++++   | ++++              | +++               | ++                | +                 | +       |
| 0.02 mg/ml                        | ++++  | +++<br>+ | +++<br>+ | +++<br>+ | +++    | +++               | ++                | ++                | ++                | ++      |
| - Ve Control                      | ++++  | +++<br>+ | +++<br>+ | +++<br>+ | ++++   | +++               | +++               | +++               | ++                | ++      |
| +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               | +++<br>+  | ++++  | +++    | ++++   | ++     | ++<br>20%<br>dead | +<br>75%<br>dead | +<br>80%<br>dead | +<br>95%<br>dead | APD     |
| 0.16 mg/ml              | +++<br>+  | ++++  | ++++   | +++    | ++     | ++<br>20%<br>dead | +<br>50%<br>dead | +<br>70%<br>dead | +<br>90%<br>dead | APD     |

|               |          |      |      |      |      |                    |                    |                   |    |              |     |
|---------------|----------|------|------|------|------|--------------------|--------------------|-------------------|----|--------------|-----|
| 0.08<br>mg/ml | +++<br>+ | ++++ | ++++ | ++++ | ++++ | ++<br>20%<br>dead  | +++<br>40%<br>dead | ++<br>75%<br>dead | +  | 90%<br>dead  | APD |
| 0.04<br>mg/ml | +++<br>+ | ++++ | +++  | +++  | +++  | ++<br>20 %<br>dead | ++<br>20 %<br>dead | ++ 20<br>%        | +  | 20 %<br>dead | +   |
| 0.02<br>mg/ml | +++<br>+ | ++++ | ++++ | +++  | +++  | +++                | ++<br>10%<br>dead  | ++<br>10%<br>dead | +  | 20%<br>dead  | +   |
| -Ve Cont.     | +++<br>+ | ++++ | ++++ | ++++ | ++++ | +++                | +++                | +++               | ++ | ++           | ++  |
| +Ve<br>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 *Trypanosomabruceibrucei***

| Conc. of<br>NBF | Observations Made Within Time Intervals (minutes) |       |               |                   |              |             |           |             |            |            |
|-----------------|---|-------|---------------|-------------------|--------------|-------------|-----------|-------------|------------|------------|
|                 | 0<br>Min.   | 5 Min | 15 Min        | 30<br>Min         | 45<br>Min    | 60<br>Min.  | 75<br>Min | 90<br>Min   | 105<br>Min | 120<br>Min |
| 0.219<br>mg/ml  | ++++  | ++++  | ++50%<br>Dead | ++<br>90%<br>dead | D            | D           | D         | D           | D          | D          |
| 0.109<br>mg/ml  | ++++  | +++   | +++           | ++                | 50 %<br>Dead | 99%<br>Dead | D         | D           | D          | D          |
| 0.055<br>mg/ml  | ++++  | ++++  | ++++          | ++++              | +            | +           | +         | D           | D          | D          |
| 0.027<br>mg/ml  | ++++  | ++++  | ++++          | ++++              | +++          | ++          | +         | 80%<br>dead | D          | D          |
| -Ve<br>Control  | ++++  | ++++  | ++++          | ++++              | ++++         | ++++        | ++++      | +++         | +++        | ++         |
| +Ve<br>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 *Trypanosomabruceibrucei* (Federe strain), and the activity was concentration dependent. These findings were in agreement with the findings of Ogbole *et al.*, (2016), who also discovered that the crude leaf extract of *Corymbia torelliana* had *in vitro* activity against *Trypanosomabruceicrucei*.

The high antitrypanosomal effect of the test compounds observed was indicative of the high potency of the stem bark extract of *Corymbia torelliana* against *Trypanosomabruceicrucei*. 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 phytochemicals 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). Higher concentrations of these test samples produced faster trypanocidal effects. This shows that higher concentrations of plant compounds may contain more phytochemicals 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 very low amounts of phytochemicals 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 solutions did 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 phytochemicals 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 effects that were comparative to those produced by Diminazen acetate (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 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 availability of secondary phytochemicals like saponins, tannins, flavonoids, cardiac glycosides, steroids and terpenes which were detected in the plant, and have been known to have therapeutic properties (Adeniyi & Ayepola, 2008). These findings are also in

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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*.

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## 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.

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