Antitrypanosomal and Anthelminthic Properties of Ethanol Extracts of Carica papaya Linn. and Ceiba pentandra (L) Gaertn.

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1. Introduction

Neglected tropical diseases (NTDs) continue to pose major health threat to people living in rural and deprived urban areas of Sub-Saharan Africa [1, 2]. It is reported that most of these communities are affected by more than one NTD [3, 4]. In Sub-Saharan Africa, helminthosis and trypanosomosis are endemic affecting both humans and livestock leading to decreased productivity and severe economic loss [5–8].

Currently, there are limited therapeutic options for the treatment of helminthosis and trypanosomosis [9]. This problem is complicated by reports of emerging resistant parasitic strains which may render the few available drugs clinically ineffective in the near future [10, 11]. Additionally, there has been reports of serious side effects associated especially with current antitrypanosomal agents [12]. There is therefore a need to search for newer, safer, and cost-effective alternatives to combat these parasitic infections [13].

Traditional medicinal plants have been used over years to treat various ailments including helminthosis and trypanosomosis [14]. Some of these plants have been studied over the years and have been proven to be effective against trypanosomes [14–16] and helminths [12, 13], hence giving credence to their traditional or folkloric use. However, there are many of these plants which are yet to be studied. Some of
these plants are traditionally used to treat both helmint and trypanosome infection, hence serving as a better alternative for individuals infected with both parasites due to a higher chance of reduced undesirable side effects [17, 18]. Ahmed et al. [19] have reported that *Tetrapleuratetraperta* fruit has both antitrypanosomal and anthelminthic activity. *Carica papaya* Linn (family: Caricaceae) and *Ceiba pentandra* (L) Gaertn (family: Bombaceae) are indigenous plants that are traditionally used to treat trypanosom and helmint infection in Ghana. Traditionally, *C. papaya* is used as an anthelminthic, antifungal, and antidiarrhoea [20]. *Ceiba pentandra* is traditionally used to treat trypanosomiasis, helmintiasis, diarrhoea, and headache [21]. The aim of this study is to determine the antitrypanosomal and anthelmintic properties of these plants and also give credence to their folkloric use.

2. Materials and Methods

2.1. Plant Collection, Authentication, and Extraction. The leaves and stem barks of both *C. papaya* and *C. pentandra* were collected between May and June 2019 at Ayeduase and Asante Bekwai, respectively, in the Ashanti Region of Ghana. The plant materials were authenticated by Mr. Clifford Asare, a botanist at the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Voucher specimens were deposited at the Herbarium of the abovementioned Department and assigned voucher specimen numbers as indicated in Table 1.

2.2. Preparation of Plant Extracts. The plant materials were washed under running tap water and air-dried at room temperature (28 to 32°C) for 7 to 14 days to a constant weight. They were then milled to powder. An amount of 400 g of each plant material was separately macerated in 70% v/v ethanol for 72 h at a temperature of 28°C. The suspension was filtered using Whatman filter paper (number 1) (Sigma-Aldrich, Michigan, USA), and the filtrate was evaporated to dryness at 40°C using a rotary evaporator (Buchi, Switzerland). The extract was then stored in a fridge at 4°C.

2.3. In Vitro Antitrypanosomal Study

2.3.1. In Vitro Culture of T. b. brucei. Five millilitres of enriched Iscove’s Modified Dulbecco’s medium (IMDM) (ThermoFisher Scientific, USA) were dispensed into 5 mL culture flasks, and 50 μL of GUT at 3.1 strain of the bloodstream form of *T. b. brucei* parasites was added and incubated at 37°C and 5% carbon dioxide for 24 hours. Parasitaemia was observed and counted daily using Neu- bauer’s chamber (Hauser Scientific, USA) till they reached a level of confluence (usually at 10⁶ parasites/mL). Fifty microlitres of parasites suspension were subcultured into new culture flask containing fresh IMDM as described in a previous study [22].

2.3.2. Evaluation of In Vitro Antitrypanosomal Activity of Plant Extracts. GUT 3.1 strain of the bloodstream form of *T. b. brucei* parasites were cultured in Iscove’s Modified Dulbecco’s medium (IMDM) as described by Yabu et al. [22]. Stock solutions of 3200 μg/mL of the extracts were prepared. Fifty microlitres of the solutions were transferred into their respective 96-well microtitre plate containing equal volumes of IMDM to give a concentration of 1600 μg/mL per well. Fifty microlitres of *Trypanosoma* parasites (3 × 10⁵ cells/mL) were then added to the wells and serially diluted (two-fold dilutions) to produce concentrations ranging from 12.5 to 800 μg/mL. *Coptis japonica* was used as positive control because it exhibits very strong antitrypanosomal activity [22, 23], while parasite cultures in IMDM only were used as negative control. The plates were then incubated for 24 h at 37°C, and 5% carbon dioxide after which 10 μL of AlamarBlue dye (G-Biosciences, USA) was added to each well and further incubated for 24 h under the same conditions. The plates were then read at 570 nm with a spectrophotometer [24]. The experiment was carried out in triplicate.

2.3.3. Determination of Cytotoxicity of Plant Extracts. Cytotoxic activity of the plant extracts was determined using the Alamar blue™ redox-based microplate assay as described by O’Brien et al. [25] using Jurkat cell lines. Stock solutions (10 mg/mL) of the extracts were prepared and serially diluted in Roswell Park Memorial Institute (RPMI) medium to give a concentration of 400 μg/mL. Fifty microlitres (50 μL) each of the respective 400 μg/mL extracts were measured and added to equal volume to achieve a concentration of 200 μg/mL for each extract. Fifty microlitres of the Jurkat cell lines (5 × 10⁴ cells/mL) were then seeded with the extracts and serially diluted to produce varying concentrations ranging from 1.56 to 100 μg/mL. The plates were incubated for 24 h at 37°C and 5% carbon dioxide after which 10 μL of AlamarBlue dye (G-Biosciences, USA) was added to each well and further incubated for 24 h under the same conditions. The absorbance was then read at 570 nm and used to calculate the IC₅₀. The experiment was carried out in triplicate. The selectivity index (SI), which measures the effectiveness and safety of a drug, was calculated as the ratio of the IC₅₀ of mammalian cell lines to the IC₅₀ of parasites.

2.4. In Vitro Anthelminthic Study

2.4.1. Determination of Anthelminthic Activity of Plant Extracts. This in vitro anthelminthic test was conducted on adult *Pheretima posthuma* using the method described by Bharathi et al. [26]. The worms (measuring between 0 and 7.0 cm in length and 0.2 to 0.3 cm in width) were collected from the Wiwi River behind the Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. The worms were first washed with normal saline to remove all debris. Stock solutions of 40 mg/mL of the extracts were prepared and serially diluted to obtain concentrations ranging from 0.625 to 10 mg/mL. Three worms of equal size were exposed...
to each concentration in Petri dish and monitored hourly for paralysis or death for a maximum period of 8 h. Albendazole (10 mg/mL) and normal saline served as positive and negative controls, respectively. Paralysis was noted when a decrease in the twisting movement was observed except when the worms were shaken vigorously or pricked with a pin. Death was concluded when the worms neither moved when shaken vigorously nor recovered when placed in the normal saline. The assay was performed in triplicate.

2.4.2. Determination of the Influence of Aqueous Extract of Plants on the Anthelmintic Activity of Albendazole. The effect of the extracts on anthelmintic activity of albendazole was determined as described by Adu et al. [27]. Subminimal inhibitory concentrations (depending on the MIC of the plant extract) of each plant extract were prepared in normal saline and used as solvent to prepare 10 mg/mL stock solution of albendazole which was diluted serially to obtain the different concentrations ranging from 2.5 to 10 mg/mL. Normal saline was used as a negative control. Three adult *P. posthuma* worms were exposed to 50 mL of the respective concentrations in a Petri dish. The worms were observed hourly over an 8 h period for paralysis or death. The experiment was carried out in triplicate.

2.5. Phytochemical Screening. The extracts were screened for the presence of secondary phytoconstituents such as tannins, saponins, flavonoids, reducing sugars, alkaloids, triterpenoids, and steroids using standard methods [24–26].

2.6. GC-MS Analysis. GC-MS examination of the samples was conducted using the PerkinElmer GC Clarus 580 Gas Chromatograph interfaced with the PerkinElmer Mass Spectrometer (Clarus SQ 8 S) with ZB-5HTMS (5 per cent diphenyl/95 per cent dimethyl polysiloxane) coupled with the capillary column (30 × 0.25 μm ID × 0.25 μm DF). Helium was used as a carrier at a steady flow rate of 1 mL/min. The temperature of the injector was set at 250°C, and the oven temperature was set at 100°C for 2 min and gradually increased to 280°C for 22 min.

2.7. Data Analysis. Data were analysed using GraphPad Prism version 6.0 for Windows. One-way ANOVA followed by Dunnett’s post hoc test and multiple t-test were employed in the analysis of data.

### Table 1: Medicinal plants collected for the study.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Part used</th>
<th>Voucher specimen number</th>
<th>Location</th>
<th>Geographic location</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. papaya</em></td>
<td>Leaves</td>
<td>KNUST/HM1/2020/L005</td>
<td>Ayeduase</td>
<td>Longitude, 6.4524 Latitude, -1.6277</td>
</tr>
<tr>
<td><em>C. papaya</em></td>
<td>Stem bark</td>
<td>KNUST/HM1/2020/SB007</td>
<td>Ayeduase</td>
<td>6.4524 –1.6277</td>
</tr>
<tr>
<td><em>C. pentandra</em></td>
<td>Stem bark</td>
<td>KNUST/HM1/2020/SB009</td>
<td>Asante Bekwai</td>
<td>6.6804 –1.5468</td>
</tr>
<tr>
<td><em>C. pentandra</em></td>
<td>Leaves</td>
<td>KNUST/HM1/2020/L004</td>
<td>Asante Bekwai</td>
<td>6.6804 –1.5468</td>
</tr>
</tbody>
</table>

3. Results

3.1. In Vitro Trypanocidal Activity. The half maximal inhibitory concentration (IC$_{50}$) values and selectivity index (SI) for the extracts against *T. b. brucei* are determined and summarized in Table 2. Ethanol extracts of *C. papaya* stem bark (PPSe) and leaves (PPLe) had moderate antitrypanosomal activity with IC$_{50}$ (SI) of 23.32 (0.42) μg/mL and 33.66 (0.60) μg/mL, respectively. Ethanol extracts of *C. pentandra* stem bark (CPSe) exhibited strong antitrypanosomal activity with an IC$_{50}$ of 11.70 μg/mL but weak SI of 0.53. Ethanol extract of *C. pentandra* leaves (CPLe) showed moderate antitrypanosomal activity with the IC$_{50}$ of 20.36 with weak SI of 1.05 (Table 2).

3.2. Anthelmintic Activity of Albendazole on *P. posthuma*. Albendazole exhibited a dose-dependent effect on the *P. posthuma* worms (Figures 1(a) and 1(b)). Albendazole at a concentration of 10 mg/mL significantly (*p* < 0.0001) paralyzed and killed worms after 165.23 ± 1.78 and 324.70 ± 2.85 minutes, respectively, but could not induce paralysis (0.625 and 1.25 mg/mL of albendazole) nor death (0.625, 1.25, and 2.5 mg/mL of albendazole) after the maximal exposure time (480 minutes) (Figures 1(a) and 1(b)).

3.3. Anthelmintic Activity of Crude Plant Extracts

3.3.1. Anthelmintic Activity of Ethanol Extract of *C. papaya* (PPSe). The action of PPSe on the worms was concentration-dependent, with all concentrations capable of paralyzing and killing the worms after the maximal exposure time. In comparison with the negative control, PPSe at 0.625 mg/mL substantially (*p* < 0.0001) induced paralysis at 249.61 ± 2.3 minutes and death at 363.68 ± 1.9 minutes (Figure 2). PPSe significantly (*p* < 0.001) induced paralysis after 71.88 ± 0.62 minutes and death after 231.62 ± 0.42 minutes compared to the negative control (Figures 2(a) and 2(b)).

PPLe caused no paralysis or death after the maximum exposure time (480 minutes) at all the test concentrations (0.625–10 mg/mL) which was similar to the effect of the normal saline (negative control group) on the worms (graph not shown).

3.3.2. Anthelmintic Activity of Ethanol Extract of *C. pentandra*. CPSe (10 mg/mL) significantly (*p* < 0.0001) paralyzed and killed the worms after 59.15 ± 0.94 and 161.58 ± 0.83 minutes, respectively, of exposure compared to
negative control (Figure 3). At the lowest dose of 0.625 mg/mL, it significantly \((p < 0.0001)\) paralyzed the worms after 241.86 ± 0.26 minutes when compared to the negative control (Figure 3(a)), but did not kill the worms after the maximum exposure duration (Figure 3(b)). After 480 minutes of exposure, 0.625 and 1.25 mg/mL of CPSe exhibited no lethal effect on worms (Figure 3(b)).

CPLe caused no paralysis or death after the maximum exposure time (480 minutes) at all the test concentrations (0.625–10 mg/mL) which was similar to the effect of the

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**Table 2: In vitro antitrypanosomal activities of crude extracts against *T. b. brucei*.**

<table>
<thead>
<tr>
<th>Plant species</th>
<th><em>T. b. brucei</em> IC(_{50}) (μg/mL)</th>
<th>Jurkat cell line IC(_{50}) (μg/mL)</th>
<th>Selectivity index</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Coptis japonica</em></td>
<td>23.32</td>
<td>9.89</td>
<td>2.41</td>
</tr>
<tr>
<td>PPSe</td>
<td>33.66</td>
<td>20.34</td>
<td>1.64</td>
</tr>
<tr>
<td>PPLe</td>
<td>11.70</td>
<td>6.24</td>
<td>1.89</td>
</tr>
<tr>
<td>CPSe</td>
<td>20.38</td>
<td>21.38</td>
<td>0.94</td>
</tr>
<tr>
<td>CPLe</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PPSe = ethanol extract of *Carica papaya* stem bark; PPLe = ethanol extract of *C. papaya* leaves; CPSe = ethanol extract of *Ceiba pentandra* stem bark; CPLe = ethanol extract of *C. pentandra* leaves; *Coptis japonica* = positive control.
normal saline (negative control group) on the worms (graph not shown).

3.4. Influence of Extracts on the Anthelminthic Activity of Albendazole

3.4.1. Influence of PPSe on the Anthelminthic Activity of Albendazole. After 289.45 ± 3.05 minutes of exposure, 2.5 mg/mL albendazole induced paralysis of the worms (Figure 4). When 0.1 mg/mL PPSe was combined with 2.5 mg/mL albendazole, the paralysis time was dramatically reduced to 181.37 ± 0.68 minutes when compared to albendazole alone (Figure 4(a)). When 0.5 mg/mL PPSe was combined with 2.5 mg/mL albendazole, the paralysis time further reduced significantly ($p < 0.0001$) to 153.54 ± 0.62 minutes (Figure 4(b)).

Albendazole at 2.5 mg/mL was ineffective in killing the worms, but when combined with 0.5 mg/mL PPSe, death occurred at 254.96 ± 0.99 minutes (Figure 4(d)). There was no fatality after the maximal exposure time when 2.5 mg/mL albendazole was combined with 0.1 mg/mL PPSe (Figure 4(c)). When PPSe at concentrations of 0.1 and 0.5 mg/mL was each added to higher concentrations of albendazole (5 and 10 mg/mL), paralysis and death times further reduced significantly ($p < 0.0001$) in a concentration-dependent manner compared to the negative control (Figure 4).

3.5. Influence of PPLe on the Anthelminthic Activity of Albendazole. Albendazole alone significantly ($p < 0.0001$) induced paralysis and death (but not at 2.5 mg/mL). When 5 and 8 mg/mL of PPLe were combined with each concentration (2.5, 5, and 10 mg/mL) of albendazole, the paralytic effect of albendazole was lost when 5 and 8 mg/mL of PPLe were added to each concentration of albendazole except for the 10 mg/mL albendazole in combination with 8 mg/mL of PPLe where the paralytic time was significantly ($p < 0.0001$) prolonged from 165.24 ± 1.03 to 272.90 ± 0.61 minutes (Figures 6(a) and 6(b)). When 5 and 8 mg/mL of CPLe were combined with each concentration (2.5, 5, and 10 mg/mL) of albendazole, the lethal effect of albendazole was lost after the maximum exposure time, with no significant difference ($p > 0.05$) between the treatment (albendazole plus extract) groups and the negative control group (Figures 6(c) and 6(d)).

3.6. Influence of CPLe on the Anthelminthic Activity of Albendazole. Albendazole alone significantly ($p < 0.0001$) induced paralysis and death (but not at 2.5 mg/mL). The paralytic effect of albendazole was lost when 5 and 8 mg/mL of CPLe were added to each concentration of albendazole except for the 10 mg/mL albendazole in combination with 8 mg/mL of CPLe where the paralytic time was significantly ($p < 0.0001$) prolonged from 165.24 ± 1.03 to 272.90 ± 0.61 minutes (Figures 7(a) and 7(b)). When 5 and 8 mg/mL of CPLe were combined with each concentration (2.5, 5, and 10 mg/mL) of albendazole, the lethal effect of albendazole was lost after the maximum exposure time, with no significant difference ($p > 0.05$) between the treatment (albendazole plus extract) groups and the negative control group (Figures 7(c) and 7(d)).

3.7. Influence of CPSe on the Anthelminthic Activity of Albendazole. When 1 mg/mL of CPSe was added to 2.5 and 5 mg/mL of albendazole, the paralytic effect of albendazole was lost. Additionally, the combination of 1 mg/mL of CPSe with 10 mg/mL of albendazole significantly ($p < 0.0001$) prolonged the paralytic time from 165.24 ± 1.03 to 384.94 ± 2.45 minutes (Figure 7(a)). When 2 mg/mL of CPSe was added to 5 and 10 mg/mL of albendazole, the paralytic time was significantly ($p < 0.0001$) prolonged whereas at 2.5 mg/mL, the paralytic effect of albendazole was lost (Figure 7(b)). At the same time, the lethal effect was lost when CPSe (1 and 2 mg/mL) was combined with 5 and 10 mg/mL of albendazole (Figures 7(c) and 7(d)).

3.8. Preliminary Phytochemical Screening of Plant Extracts. Preliminary phytochemical screening revealed the presence of triterpenoids in all extracts. Saponins, steroids, and reducing sugars were present in all the extracts except CPSe. Tannins were present in only PPLe and CPSe. Flavonoids were also found in PPLe and CPLe only. Only PPSe contained alkaloids (Table 3).
3.9. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis. GC-MS analysis of the most active extracts (PPSe and CPSe) led to the identification of volatile compounds that could as well contribute to their therapeutic effect. These compounds were identified through mass spectrometry and GC. The various compounds present are shown in Tables 4 and 5. The compounds 9-octadecenamide and n-hexadecanoic acid were found in both PPSe and CPSe.

4. Discussion

Traditional medicinal plants have contributed enormously to the search for new antimicrobial agents. All the plant extracts used in this study had some level of activity against T. b. brucei with IC_{50} between 20 and 50 μg/mL. According to Ohashi et al. [15], a plant extract with IC_{50} < 20 μg/mL is considered to have strong activity. The most active plant extract in our investigation was CPSe with an IC_{50} of 11.70 mg/mL. The other three extracts, namely, PPSe, PPLE, and CPLe showed moderate antitrypanosomal activity with an IC_{50} of 23.32, 33.66, and 20.38 μg/mL, respectively. The observed activity could be attributed to the secondary metabolites found in the crude extracts. Flavonoids, triterpenoids, and tannins have been reported to exhibit antitrypanosomal activity [12, 19]. Hence, the presence of tannins and triterpenoids in CPSe may account for its strong antitrypanosomal activity. Though all the other extracts contained triterpenoids and other secondary metabolites, only CPSe showed significant activity.
metabolites, the method used is a qualitative test and hence does not predict the amount of extract. Hence, the observed moderate to weak antitrypanosomal activity exhibited by these extracts may be due to low levels or trace amounts of these phytochemicals in those extracts. GC-MS analysis revealed the presence of n-hexadecanoic acid in PPSe and CPSe and stigmast-4-en-3-one in PPSe which have both been reported to possess antitrypanosomal activity against *T. b. brucei* [28]. Selective index (SI) is a ratio that measures the toxicity of a drug. Muganza et al. [16] described SI above 10 as acceptable, but all the extracts used for this study had SI below 10 which mean they may be toxic during *in vivo* use.

All the four crude extracts were as well screened against *P. posthuma* earthworms *in vitro*. *P. posthuma* was used since they have been documented to possess physical and anatomical resemblance to *A. lumbricoides* [22, 27]. The observed activity of PPSe may be due to the presence of alkaloids and saponins which have been stated by Gao-Xue et al. [29] to possess anthelminthic activity. PPLe possessed

**Figure 5**: Influence of PPLe on anthelminthic activity of albendazole. *n* = 3; values are mean ± SEM; *** *p* < 0.0001 compared to control (multiple *t*-tests); *ns* = not significant; PPLe = *C. papaya* leaves ethanol extract. (a) Paralysis time of PPLe (5 mg/mL). (b) Paralysis time of PPLe (8 mg/mL). (c) Death time of PPLe (5 mg/mL). (d) Death time of PPLe (8 mg/mL).
bioactive compounds such as tannins and flavonoids but were unable to cause death to the worms since they may be present in trace amounts and not enough to have any significant effect on the worms. CPSe was able to kill the worms at the least concentration of 0.25 mg/mL. CPSe contains tannins, but flavonoids and saponins were absent. -X_hus, the activity may be due to a high concentration or amount of tannins in CPSe.

When subminimal inhibitory concentration of the extracts was combined with albendazole, it was found that only PPSe greatly potentiated the anthelmintic effect of albendazole. The enhanced activity of albendazole could be due to presence of secondary metabolites in the PPSe that rendered the drugs more available at their sites of action thus potentiating the anthelmintic effect [25, 27, 30]. The combination of CPSe, PPLe, and CPLe inhibited the parasitic effect of albendazole on the earthworms. Research has it that some phytochemicals can form complexes with antimicrobial agents which can lead to decreased absorption, decreased affinity to the target site, and loss of biological activity [27].
Table 3: Phytochemical composition of *Carica papaya* and *C. pentandra* extracts.

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>C. papaya</th>
<th>C. pentandra</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PPLE</td>
<td>PPSe</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

Key: + = phytochemical present; − = phytochemical absent. PPSe = ethanol extract of *Carica papaya* stem-bark; PPLE = ethanol extract of *Carica papaya* leaves; CPSe = ethanol extract of *Ceiba pentandra* stem bark; CPLe = ethanol extract of *C. pentandra* leaves.
5. Conclusion

CPSe exhibited strong activity against *T. b. brucei* but weak selectivity index (SI) and may not be safe for in vivo application. All the other extracts showed moderate antitrypanosomal activity. PPSe potentiated the activity of albendazole, while CPSe, CPLe, and PPLe inhibited the anthelminthic activity of albendazole. Phytochemical screening performed on the plants revealed the presence of bioactive compounds such as tannins, alkaloids, reducing sugars, saponins, and flavonoids in the plant extracts. GC-MS analysis led to the identification of stigmast-4-en-3-one and n-hexadecanoic acid which have been reported to possess antitrypanosomal activity against *T. b. brucei*.

Data Availability

The datasets used and/or analysed during the current study are included within the article. Further clarification can be obtained from the corresponding author.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors’ Contributions

FA, CA, and YDB conceived and designed the experiment. AWO performed the experiments. AWO, TAA, and VEB analysed and interpreted the data. JOP, AAB, and SYG assisted in and provided reagents for the GC-MS studies. FA, CA, and YDB provided all other reagents and materials. VEB and TAA provided analysis tools. AWO and YDB developed the first draft of the manuscript. All authors contributed equally to the revision of manuscript to its final stage.

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References


