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Antitrypanosomal, antiplasmodial, and antibacterial activities of extracts from selected *Diospyros* and Annonaceae species

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ABSTRACT

Aim: To screen methanol extracts from root bark, leaves, and stem bark of selected plant species from the genus *Diospyros* and some Annonaceae species for antitrypanosomal, antiplasmodial, and antibacterial activities against selected test organisms.

Methods: Antitrypanosomal and antiplasmodial assays of methanol extracts from selected plant species were carried out in single concentration screens and in dose-response for active extracts. The minimum inhibitory concentration (MIC) values of selected plant extracts against selected bacterial strains were determined by microplate dilution method in sterile 96-well microtiter plates.

Results: In the dose-response antitrypanosomal assay, the most potent extracts tested exhibited activities against *Trypanosoma brucei brucei* (Lister 427 strain) with IC_{50} values ranging from 1.28 to 7.85 µg/ml, with methanol extract of *Diospyros verrucosa* stem bark being the most active with IC_{50} value of 1.28 µg/ml. In the dose-response antiplasmodial assay, three extracts exhibited activities against *Plasmodium falciparum* (strain 3D7) with IC_{50} values ranging from 4.55 to 24.22 µg/ml, with methanol extract of *Diospyros capricornuta* root bark being the most potent with IC_{50} value of 4.55 µg/ml. In the antibacterial assay, the investigated extracts exhibited a wide range of activities against *Staphylococcus aureus* [American Type Culture Collection (ATCC) strain 25923], *Bacillus cereus* (ATCC strain 11775), and *Escherichia coli* (ATCC strain 8740) with MIC values ranging from 0.00125 to 0.00625 mg/ml (more active), 0.125 to 0.500 mg/ml (moderately active), and 1.00 to 8.00 mg/ml (less active) while some extracts were inactive at the highest concentration tested of 16.00 mg/ml.

Conclusions: Methanol extracts obtained from root bark, leaves, and stem bark of selected plant species from the genus *Diospyros* and some Annonaceae species that showed good activities in antitrypanosomal, antiplasmodial, and antibacterial assays corroborate reported literature about the traditional medicinal uses of the members of genus *Diospyros* and some Annonaceae species.

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Introduction

Infectious diseases are the leading causes of death worldwide. About 14.9 million annual human deaths worldwide are caused by infectious diseases [1]. According to the World Health Organization (WHO), human African trypanosomiasis (HAT), malaria, and bacterial diseases are among the infectious diseases with the highest epidemics [2].

HAT, commonly known as sleeping sickness is a disease caused by two subspecies of extracellular protozoan parasites, namely *Trypanosoma brucei gambiense* and *T. b. rhodesiense*. The four drugs

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currently available for the treatment of human African sleeping sickness are pentamidine, suramin, melarsoprol, and eflornithine. Nifurtimox, another drug, that was introduced in the market in the 1960s for the treatment of Chagas disease (human American trypanosomiasis), is restricted to treatment of HAT in combination with other trypanocidal drugs for patients who do not respond to late stage medicines [3]. In 2009, nifurtimox–eflornithine combination therapy used for the treatment of late stage HAT, caused by *T. b. gambiense* infections, was included on the WHO essential medicines list. Despite the advancement in HAT treatment, the currently available drugs are unsatisfactory for various reasons including unacceptable toxicity, poor efficacy, undesirable route of administration, and drug resistance [4]. This inspires the need to carry out ethnopharmacological investigations towards identification of possible active plant extracts that may be investigated for the development of new antitrypanosomal pharmaceuticals.

Malaria is a disease caused by infection of red blood cells with protozoan parasites of the genus *Plasmodium* inoculated into the human host by the blood-feeding female *Anopheles* mosquitoes. Treatment of malaria is also affected by drug resistance. If different drugs with different mechanisms of resistance are used together, the emergence and spread of resistance can be limited [5]. As a result, combination therapy is used to reduce the development of drug resistance, and most countries with *P. falciparum* malaria have adopted artemisinin-based combination therapies (ACTs) as first-line medications. ACTs have been estimated to reduce malaria mortality in children aged 1–23 months by 99% and in children aged 24–59 months by 97% [6]. Despite the use of combination therapy, *Plasmodium falciparum* resistance to ACTs has been detected in five countries in the Greater Mekong sub-region. Drug resistance has been documented for all classes of antimalarial chemotherapies and is a major threat to malaria control efforts [5]. Thus, the discovery of antiplasmodial active plant extracts for potential drug development is important to increase the number of alternative medicines available.

The Gram-positive bacterium *Staphylococcus aureus* is the causative agent of skin inflammations, intestinal infections, and pneumonia. The emergence of strains of *S. aureus* resistant to some antibiotics such as methicillin has been documented [7,8]. *Bacillus cereus*, a Gram-positive bacterium that causes two types of gastrointestinal diseases (the diarrheal and the emetic syndromes) together with the Gram-negative bacterium, *Escherichia coli* which result in three clinical syndromes (namely diarrheal disease, urinary tract infections, and meningitis) is also resistant to available chemotherapies [9,10]. Thus, the development of antibacterial active plant extracts that can be used for the discovery of antibacterial drugs is also necessary.

Medicinal plants provide a reliable source of biologically active compounds; and thus, a search for extracts that are active against parasitic protozoans such as trypanosomes and plasmodia as well as pathogenic bacteria could aid the discovery of drugs. The genus *Diospyros* is known for various traditional medicinal uses including for the treatment of HAT, malaria, headache, diarrhea, dysentery, stomach ache, and inflammatory conditions [11–18]. According to interviewed natives during the field excursion, *Diospyros natalensis* is used as a herbal remedy for the treatment of fever and internal body pain [19]. Plant species in the family Annonaceae are also known for their uses as traditional medicines for the treatment of various diseases. *Greenwayodendron suaveolens* is used as a herbal remedy for the treatment of malaria and helminthiasis [20,21]. The genus *Uvariodendron* is used as a traditional medicine for the treatment of skin inflammation and liver disorders [22]. The root of *Uvaria tanzaniae* is used as a herbal remedy for the control of fever [23]. In this work, therefore, we report the *in vitro* antitrypanosomal, antiplasmodial, and antibacterial activities of extracts from selected *Diospyros* and some Annonaceae species.

Materials and Methods

Collection of plant materials

The root bark, leaves, and stem bark of *Diospyros* species selected for the study were collected in Tanzania as follows: *D. bussei* Gurke in June 2014 at Koloha-Kwakihande, Mkange village in Bagamoyo district. GPS location: S 06°03'24.0", E 038°36'21.6"; elevation 196 m. *Diospyros natalensis* (Harv.) Brenan in May 2014 at Manolo Forest Reserve in Lushoto District. GPS location: S 04°39'02.3", E 038°12'36.0". *Diospyros squarrosa* Klotzsch in May 2014 at Madala, Tuliani Village in Handeni District. GPS location: S 05°40'18.7", E 038°05'20.4"; elevation 595 m. *Diospyros verrucosa* Hiern in June 2014 at Gongo Village in Bagamoyo District. GPS location: S 06°09'57.8", E 038°37'33.1"; elevation 302 m. *D. capricornuta* F. White in June 2014 at Pugu forest reserve in Kisarawe District. GPS location: S 06°53'28.4", E 039° 05'56.3"; elevation 269 m.

Diospyros kabuyeana F. White in June 2014 at Pugu Forest Reserve in Kisarawe District. GPS location: S 06°54'26.2", E 039°05'51.1" (Fig. 1). The plant species were identified in the field and confirmed at the herbarium of the Department of Botany, University of Dar es Salaam where voucher specimens FMM 3663, FMM 3661, FMM 3660, FMM 3664, FMM 3667, and FMM 3669 of *D. bussei*, *D. natalensis*, *D. squarrosa*, *D. verrucosa*, *D. capricornuta*, and *D. kabuyeana*, respectively, are preserved.

The root bark, leaves, and stem bark of the species selected for the study from the family Annonaceae were collected in Tanzania as follows: *Greenwayodendron suaveolens* subs. *usambaricum* Verdc in October 2015 at River Mombo Forest, Kisiwani Village in Muheza District. GPS location: 37 M 0461716 Universal Transverse Mercator (UTM) 9434638; elevation 961 m. *Uvaria tanzaniae* Verdc in October 2015 at Fanusi in Kisiwani Village, Muheza District. GPS location: 37 M 0464474 UTM

Figure 1. Representatives of plant species collected (a) Diospyros bussei; (b) Diospyros capricornuta; (c) Diospyros kabuyeana; (d) Diospyros natalensis; (e) Diospyros squarrosa; and (f) Diospyros verrucosa.

9434330. *Uvariodendron usambarense* R.E.Fr. in October 2015 at River Mombo Forest, Kisiwani Village in Muheza District. GPS location: 37 M 0461716 UTM 9434638; elevation 961 m. The selected species were identified in the field and confirmed at the herbarium at the Department of Botany, University of Dar es Salaam where voucher specimens FMM 3708, FMM 3711, and FMM 3707 of *G*. *suaveolens* subs. *usambaricum*, *U*. *tanzaniae*, and *U*. *usambarense*, respectively, are preserved.

Extraction of plant materials

The air-dried and pulverized root bark, leaves, and stem bark of plant species selected for the study (20 g each) were each extracted using methanol at room temperature for 48 hours. Concentration of extracts was done by removal of solvent under reduced pressure using a rotary evaporator to afford the crude extracts for the biological assays. Methanol was used due to its polarity to mimic the use of water in preparation of decoctions of traditional medicines.

In vitro *antitrypanosomal assay*

The *in vitro* antitrypanosomal assay of the methanol extracts of selected plant species was carried out at the Centre for Chemico- and Biomedicinal Research, Rhodes University in South Africa in 2016. The method described by Hirumi and Hirumi [24] was used to culture parasites.

Trypanosoma brucei brucei trypomastigotes (Lister 427 strain) were cultured at 37°C in a 5% CO_2 incubator in Iscove's modified Dulbecco's medium containing 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 4 mM L-glutamine (Lonza). The medium was further supplemented with 10% fetal calf serum, penicillin/streptomycin sulfate (100 units/ml and 0.1 mg/ml, respectively), 1 mM hypoxanthine, and Hirumi's modified Iscove's medium 9 (1.5 mM cysteine, 1.25 mM pyruvic acid, 0.1 mM cytosine, 0.15 mM thymidine, 0.1 mM uracil, 0.05 mM bathocuproinedisulfonic acid, and 0.2 mM 2-mercaptoethanol).

To assess the antitrypanocidal activity in a single concentration screen, extracts were added to *in vitro* cultures of *T*. *b*. *brucei* placed in 96-well plates at a fixed concentration of 25 μg/ml in duplicate followed by incubation at 37°C for 48 hours. Residual parasite viability in the wells was determined by adding 20 μl resazurin solution (0.135 mg/ml in phosphate buffered saline) and incubating for an additional 2–4 hours. Reduction of resazurin to resorufin by viable parasites was assessed by measuring fluorescence (excitation 560 nm, emission 590 nm) in a SpectraMax M3 plate reader. Fluorescence readings were converted to percentage parasite viability relative to the average readings obtained from untreated control wells. Results were expressed as percentage parasite viability against extracts in concentration of 25 μg/ml.

Extracts that reduced parasite viability to <25% (inhibition > 75%) were considered for further testing in a dose-response assay. To determine the antitrypanocidal potency of active extracts, *in vitro* cultures of *T*. *b*. *brucei* were added to serial dilutions of extracts in 96-well plates and incubated for 48 hours. The 50% inhibitory concentration (IC_{50}) values were determined by plotting percentage viability *vs*. log [extract] and performing non-linear regression using GraphPad Prism (version 5.02) software. Pentamidine (an existing drug for the treatment of trypanosomiasis) was used as a positive control drug standard and yielded an IC_{50} value of 0.5 nM.

In vitro *antiplasmodial assay*

The *in vitro* antiplasmodial assay of methanol extracts was carried out at the Centre for Chemico- and Biomedicinal Research, Rhodes University in South Africa in 2016. The method described by Makler and Hinrichs [25] was used to determine antiplasmodial activities of methanol extracts in a single concentration screen and in dose-response for active extracts.

A *Plasmodium falciparum* chloroquine-sensitive strain (3D7) was cultured in Roswell Park Memorial Institute medium 1640 containing 25 mM HEPES and 2 mM L-glutamine (Lonza). The medium was further supplemented with 0.5% (w/v) Albumax II (Thermo Fisher Scientific), 22 mM glucose, 0.65 mM hypoxanthine, 0.05 mg/ml gentamicin, and 2%–4% (v/v) human erythrocytes. Cultures were maintained at 37°C under an atmosphere of 5% CO_2, 5% O_2, and 90% N_2.

To assess antiplasmodial activity in a single concentration screen, extracts were added to parasite cultures (adjusted to 2% parasitaemia, 1% haematocrit) in 96-well plates at a fixed concentration of 25 μg/ml in duplicate followed by incubation at 37°C for 48 hours. Parasite lactate dehydrogenase enzyme activity in the individual wells was subsequently determined by removing 20 μl of the parasite cultures and mixing it with 125 μl colorimetric substrate solution containing 44 mM tris (hydroxymethyl) aminomethane (pH 9), 0.18 M L-lactic acid, 0.13 mM acetylpyridine adenine

dinucleotide, 0.39 mM nitrotetrazolium blue chloride, 0.048 mM phenazine ethosulfate, and 0.16% (v/v) Triton X-100. Color development was monitored by measuring absorbance at 620 nm in a SpectraMax M3 plate reader (Molecular Devices). Absorbance values were converted to percentage parasite viability relative to untreated control cultures after subtracting background absorbance readings obtained from wells containing erythrocytes alone (i.e., without parasites). Wells without extracts and without parasites, thus, acted as positive and negative control sets. Results were expressed as percentage parasite viability against extracts in concentration of 25 µg/ml.

Extracts that reduced parasite viability to <25% (inhibitions > 75%) were considered for further testing in a dose-response assay. To determine the antiplasmodial potency of active extracts, parasite cultures (adjusted to 2% parasitaemia, 1% haematocrit) were added to serial dilutions of extracts in 96-well plates in duplicate followed by incubation at 37°C for 48 hours. As described above, absorbance was measured at 620 nm and percentage parasite viability in extract-treated wells calculated relative to untreated control wells, after subtracting background absorbance readings obtained from non-parasitized control wells. The IC_{50} values were determined by plotting percentage viability *vs*. log [extract] and performing non-linear regression using GraphPad Prism (version 5.02) software. For comparative purposes, chloroquine (an antimalarial drug) was used as standard and produced an IC_{50} value of 2.5 nM.

In vitro *antibacterial assay*

In vitro antibacterial screening of methanol extracts of root bark, leaves, and stem bark from selected plants was carried out at the Department of Pharmacy and Pharmacology in the Faculty of Health Sciences, University of the Witwatersrand in 2016.

Solvents (acetone and dimethyl sulfoxide) were supplied by Merck (Darmstadt, Germany). Ciprofloxacin and *p*-iodonitrotetrazolium (INT) chloride were purchased from Sigma-Aldrich (Missouri, USA). Ninety-six well microtiter plates were supplied by AEC-Amersham (Johannesburg, South Africa). Tryptone Soya broth was obtained from Thermo Fisher Scientific (Waltham, USA). The three pathogens namely *Staphylococcus aureus* (ATCC strain 25923), *Bacillus cereus* (ATCC strain 11775), and *Escherichia coli* (ATCC strain 8740) were supplied by Davies Diagnostics (Johannesburg, South Africa).

Bacterial strains were cultured in Tryptone Soya broth media. Tryptone Soya broth (30 g) suspended in 1 L of distilled water was autoclaved at 121°C in 30 minutes. The mixture was left to cool to room temperature. The media (20 ml) were transferred into each of the sterile culturing test tubes which were then separately inoculated with *S*. *aureus*, *B*. *cereus*, and *E*. *coli*, respectively. Test tubes containing media (Tryptone Soya broth) and inoculum were incubated at 37°C overnight. The bacterial cultures were observed after 24 hours of growth; and thus, ready for antibacterial assays.

The minimum inhibitory concentration (MIC) values of selected plant extracts against the aforementioned bacterial strains were determined by microplate dilution method in sterile 96-well microtiter plates [26]. The initial concentrations of stock solutions of plant extracts and ciprofloxacin (positive control) were prepared to 32.00 and 0.01 mg/ml, respectively. Plant extracts were dissolved using either acetone or 50% dimethyl sulfoxide/water (when samples did not dissolve in acetone) and ciprofloxacin using sterile water.

Each bacterial culture obtained after 24 hours of incubation at 37°C was diluted in two subsequent dilutions. The first dilution was carried out in 1:10 followed by the second dilution in 1:100. The resulting culture after the second dilution was placed in each of serially diluted 96-well microtiter plates (100 µl/well) (containing extracts at various concentrations) for inoculation with respective bacterial strains. Inoculated microtiter plates were then incubated at 37°C for 24 hours.

To determine MIC values of extracts, 40 µl (200 µg/ml) of *p*-INT chloride solution was added into inoculated wells and plates were examined after 4 hours (guided by a column for positive control). The MIC value of each extract was read at the lowest concentration where a marked reduction in color formation (purple/pink) due to bacterial growth inhibition was noted.

Results and Discussion

In vitro *antitrypanosomal activity*

The *in vitro* antitrypanosomal activities of methanol extracts of root bark, leaves, and stem bark of selected plant species were obtained by screening extracts against *Trypanosoma brucei brucei* in a single concentration screen at 25 µg/ml. Results (Table 1) were obtained as percentage inhibition of the test organism. Fifteen of the twenty one extracts inhibited the growth of the parasite by greater

than 75% (Table 1), and were considered for the dose-response assay to determine IC_{50} values by serial dilutions.

In the dose-response assay, results were obtained as percentage viability of the test organism against logarithm of sample concentration (µg/ml) (Fig. 2). The IC_{50} values of tested samples are presented in Table 1. The tested extracts exhibited IC_{50} values ranging from 1.28 to 7.85 µg/ml. Extracts which showed high activities are *Diospyros verrucosa* stem bark (DVSM) methanol extract (IC_{50}: 1.28 µg/ml), *Diospyros capricornuta* root bark (DCRM) methanol extract (IC_{50}: 1.56 µg/ml), and *Uvaria tanzaniae* root bark (UTRM) methanol extract (IC_{50}: 2.12 µg/ml). Others were *Diospyros verrucosa* root bark (DVRM) methanol extract (IC_{50}: 2.23 µg/ml), *Diospyros natalensis* stem bark (DNSM) methanol extract (IC_{50}: 2.85 µg/ml), and *Diospyros verrucosa* leaves (DVLM) methanol extract (IC_{50}: 2.99 µg/ml). Most of these extracts from the genus *Diospyros* showed good activities compared to the literature data for *Diospyros mespiliformis* leaves which exhibited antitrypanosomal activity against *Trypanosoma brucei brucei* at the MIC value of 500 µg/ml [17]. These extracts, together with other samples tested in a dose-response antitrypanosomal assay, could potentially contain active constituents against *T. b. brucei*. Thus, these findings concur with ethnomedicinal uses of some members of the genus *Diospyros* for the treatment of HAT.

In vitro *antiplasmodial activity*

The antiplasmodial activities of methanol extracts of root bark and stem bark of the selected plant species were determined by screening extracts against a chloroquine sensitive strain of *Plasmodium falciparum* (3D7) at a single concentration of 25 µg/ml. Results (Table 2) were obtained as percentage inhibition of the test organism. In this case, only three extracts inhibited parasite growth by more than 75%, and were considered for dose-response assay to determine IC_{50} values by serial dilutions.

In dose-response antiplasmodial assay, results were obtained as percentage viability of the test organism against logarithm of sample concentration (µg/ml) (Fig. 3). The IC_{50} values of tested samples in dose-response are presented in Table 2. The studied extracts exhibited activities with IC_{50} values

Table 1. Antitrypanosomal activities of methanol extracts from root bark, leaves, and stem bark of selected plant species.

Sample	Extract	% Inhibition at 25 µg/ml ± SD	IC_{50} (µg/ml)
Diospyros bussei Gurke leaves (Ebenaceae)	DBLM	70.6 ± 7.3	NT
Diospyros bussei root bark	DBRM	65.7 ± 2.7	NT
Diospyros bussei stem bark	DBSM	66.0 ± 4.0	NT
Diospyros capricornuta F. White leaves (Ebenaceae)	DCLM	73.5 ± 5.3	NT
Diospyros capricornuta root bark	DCRM	81.6 ± 0.3	1.56
Diospyros capricornuta stem bark	DCSM	74.1 ± 7.1	NT
Diospyros kabuyeana F. White leaves (Ebenaceae)	DKLM	81.0 ± 0.5	3.32
Diospyros kabuyeana stem bark	DKSM	79.3 ± 1.7	NT
Diospyros natalensis (Harv.) Brenan leaves (Ebenaceae)	DNLM	82.6 ± 1.5	3.74
Diospyros natalensis root bark	DNRM	80.5 ± 0.3	3.02
Diospyros natalensis stem bark	DNSM	78.3 ± 0.6	2.85
Diospyros squarrosa Klotzsch root bark (Ebenaceae)	DSRM	83.2 ± 1.5	5.38
Diospyros verrucosa Hiern leaves (Ebenaceae)	DVLM	81.1 ± 0.4	2.99
Diospyros verrucosa root bark	DVRM	79.3 ± 0.9	2.23
Diospyros verrucosa stem bark	DVSM	78.3 ± 0.7	1.28
Greenwayodendron suaveolens subs. *usambaricum* Verdc root bark (Annonaceae)	GSRM	79.4 ± 4.8	7.85
Greenwayodendron suaveolens subs. *usambaricum* stem bark	GSSM	77.5 ± 1.4	3.54
Uvaria tanzaniae Verdc root bark (Annonaceae)	UTRM	83.5 ± 0.5	2.12
Uvariodendron usambarense R.E.Fr. Leaves (Annonaceae)	UULM	82.4 ± 0.1	4.71
Uvariodendron usambarense root bark	UURM	83.3 ± 0.1	3.45
Uvariodendron usambarense stem bark	UUSM	83.7 ± 0.1	4.08
Pentamidine (positive control), IC_{50}			0.000509 µM

SD = standard deviation.
Note: codes abbreviations; first letter (generic name), second letter (specific name), third letter (part of plant collected, L = leaves, S = stem bark, and R = root bark), the last letter "M" methanol (solvent used for extraction), NT = not tested.

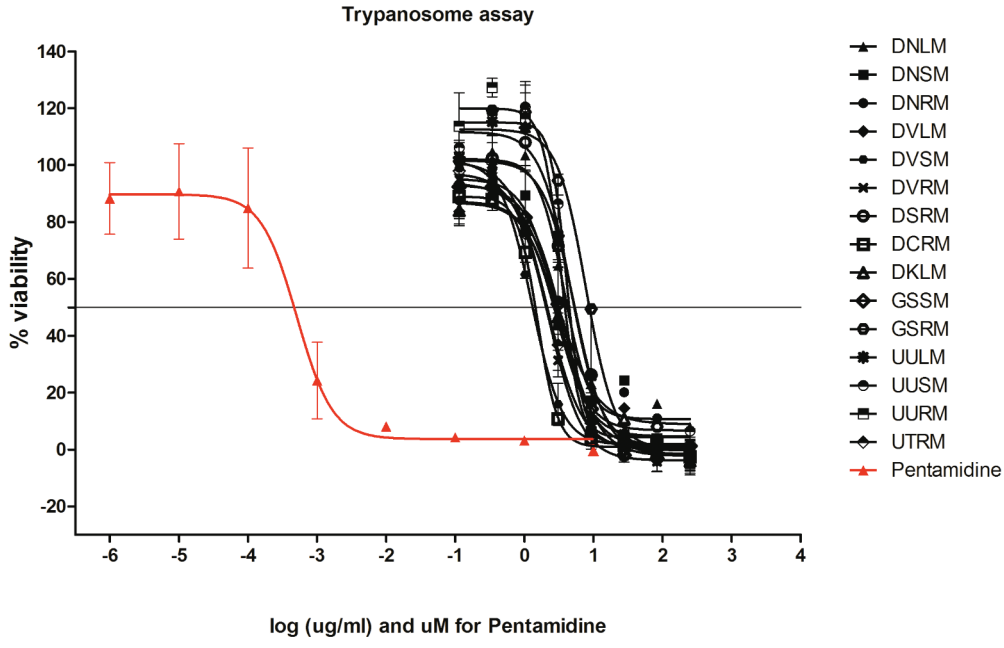

Figure 2. Dose-response antitrypanosomal activities of selected active methanol extracts from root bark, leaves, and stem bark of plant species investigated.

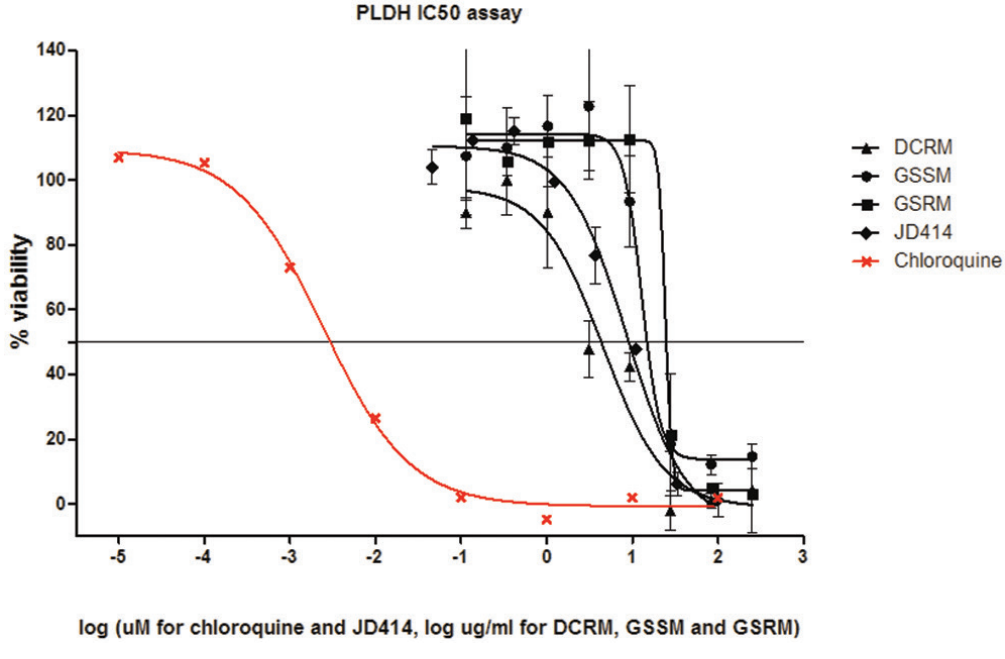

Figure 3. Dose-response antiplasmodial activities of selected active methanol extracts from stem bark and root bark of plant species studied.

Table 2. Antiplasmodial activities of methanol extracts from root bark and stem bark of selected plant species.

Sample	Extract	% Inhibition at 25 µg/ml ± SD	IC_{50} (µg/ml)
Diospyros capricornuta root bark	DCRM	85.6 ± 1.8	4.55
Greenwayodendron suaveolens subs. *usambaricum* root bark	GSRM	100.0 ± 3.2	24.22
Greenwayodendron suaveolens subs. *usambaricum* stem bark	GSSM	83.6 ± 5.7	12.89
Chloroquine (positive control), IC_{50}			0.002454 µM

SD = standard deviation.
Note: codes abbreviations; first letter (generic name), second letter (specific name), third letter (part of plant collected, S = stem bark, and R = root bark), the last letter "M" methanol (solvent used for extraction).

ranging from 4.55– to 24.22 µg/ml. Among the three samples tested in the dose-response assay, DCRM methanol extract exhibited the best activity with an IC_{50} value of 4.55 µg/ml. DCRM methanol extract exhibited good antiplasmodial activity compared to the literature data for *D. melanoxylon* which exhibited antiplasmodial activity against *Plasmodium falciparum* at IC_{50} value of 29 µg/ml [27]. Extracts investigated in the dose-response antiplasmodial assay could potentially contain lead compounds which are active against *Plasmodium falciparum*. Thus, the findings reported in this article concur with ethnobotanical uses of some members of the genus *Diospyros* and the family Annonaceae for the treatment of malaria.

In vitro *antibacterial activity*

Results for antibacterial assay were obtained as MIC values of the investigated samples in mg/ml per pathogen. The investigated extracts exhibited activities against the tested organisms with MIC values ranging from 0.00125 to 0.00625 mg/ml (more active), 0.125 to 0.500 mg/ml (moderately active), 1.00 to 8.00 mg/ml (less active), and some were inactive at the highest concentration tested of 16.00 mg/ml (Table 3).

UTRM methanol extract exhibited promising activities against *Staphylococcus aureus* and *Bacillus cereus* with MIC values of 0.00125 and <0.00625 mg/ml, respectively (Table 3). *Greenwayodendron suaveolens* subs. *usambaricum* root bark (GSRM) methanol extract and *Uvariodendron usambarense* stem bark (UUSM) methanol extract both exhibited potent activities against *B. cereus* with MIC values of <0.00625 mg/ml (Table 3). DVSM methanol extract and DVRM methanol extract both showed good activities against *Escherichia coli* with MIC values of <0.00625 mg/ml (Table 3). DVSM methanol extract and *Diospyros verrucosa* root bark methanol extract both exhibited good activities against *E. coli* compared to the literature data for *Diospyros melanoxylon* methanol bark extract which exhibited antibacterial activity against *E. coli* at MIC value of 3.0 mg/ml [28]. For samples which exhibited activities in MIC values of <0.00625 mg/ml, the amounts of the samples available during antibacterial assay were not enough to reach the end point.

Diospyros bussei leaves (DBLM) methanol extract, *D. bussei* stem bark (DBSM) methanol extract,

Table 3. Antibacterial activities of methanol extracts from root bark and stem bark of selected plant species.

Sample	Extract	MIC in mg/ml per pathogen (test organism)		
		***Staphylococcus aureus* (ATCC 25923)**	***Bacillus cereus* (ATCC 11775)**	***Escherichia coli* (ATCC 8740)**
Diospyros bussei leaves	DBLM	8.00	2.00	0.125
Diospyros bussei root bark	DBRM	NA	NA	0.500
Diospyros bussei stem bark	DBSM	NA	2.00	0.125
Diospyros capricornuta leaves	DCLM	0.250	2.00	1.00
Diospyros capricornuta root bark	DCRM	4.00	0.125	1.00
Diospyros capricornuta stem bark	DCSM	2.00	4.00	1.00
Diospyros kabuyeana leaves	DKLM	8.00	4.00	0.125
Diospyros kabuyeana stem bark	DKSM	NA	1.00	0.125
Diospyros natalensis leaves	DNLM	0.250	1.00	0.500
Diospyros natalensis root bark	DNRM	NA	NA	1.00
Diospyros natalensis stem bark	DNSM	NA	NA	0.250
Diospyros squarrosa leaves	DSLM	NA	4.00	0.250
Diospyros squarrosa root bark	DSRM	1.00	4.00	NA
Diospyros squarrosa stem bark	DSSM	NA	NA	0.500
Diospyros verrucosa leaves	DVLM	1.00	2.00	0.500
Diospyros verrucosa root bark	DVRM	NA	0.500	<0.00625
D. verrucosa stem bark	DVSM	NA	0.500	<0.00625
Greenwayodendron suaveolens subs. *usambaricum* root bark	GSRM	1.00	<0.00625	NA
Uvaria tanzaniae root bark	UTRM	0.00125	<0.00625	NA
Uvariodendron usambarense leaves	UULM	8.00	0.500	0.500
Uvariodendron usambarense stem bark	UUSM	4.00	<0.00625	NA
Ciprofloxacin (positive control)	—	0.0025	0.00008	0.00063
50% Acetone/H_2O (negative control)	—	NA	NA	NA
50% DMSO/H_2O (negative control)	—	NA	NA	NA

Note: codes abbreviations; first letter (generic name), second letter (specific name), third letter (part of plant collected, L = leaves, S = stem bark, and R = root bark), the last letter "M" methanol (solvents used for extraction); NA = no activity.

Diospyros kabuyeana leaves (DKLM) methanol extract, and *D. kabuyeana* stem bark (DKSM) methanol extract exhibited reasonable activities against *Escherichia coli* with MIC values of 0.125 mg/ml. DCRM methanol extract and *Diospyros natalensis* leaves (DNLM) methanol extract showed moderate activities against *Bacillus cereus* and *Staphylococcus aureus* with MIC values of 0.125 and 0.250 mg/ml, respectively. DNSM methanol extract and *Diospyros squarrosa* leaves (DSLM) methanol extract both exhibited modest activities against *Escherichia coli* with MIC values of 0.250 mg/ml.

DVRM methanol extract, DVSM methanol extract, and *Uvariodendron usambarense* leaves (UULM) methanol extract exhibited modest activities against *Bacillus cereus* with MIC values of 0.500 mg/ml. *Diospyros bussei* root bark (DBRM) methanol extract, DNLM methanol extract, *Diospyros squarrosa* stem bark (DSSM) methanol extract, DVLM methanol extract, and UULM methanol extract showed moderate activities against *Escherichia coli* with MIC values of 0.500 mg/ml. Extracts which showed good antibacterial activities could potentially contain constituents which are active against respective bacterial strains. Thus, these results concur with ethnobotanical uses of some members of the genus *Diospyros* and the family Annonaceae for the treatment of bacterial diseases.

Conclusions

Methanol extracts investigated in *in vitro* antitrypanosomal, antiplasmodial, and antibacterial assays that showed good activities corroborate reported literature about the traditional medicinal uses of the genus *Diospyros* (Ebenaceae) and some Annonaceae species from which plant species investigated were selected for the study. Thus, the results provide a rational support for the use of the selected plant species in traditional medicine. The findings warrant further phytochemical investigations for potential lead compounds from plant extracts that showed good antitrypanosomal, antiplasmodial, and antibacterial results. The interesting plants for future antitrypanosomal investigations are *Diospyros capricornuta*, *D. kabuyeana*, *D. natalensis*, *D. squarrosa*, *D. verrucosa*, *Greenwayodendron suaveolens* subs. *usambaricum*, *Uvaria tanzaniae*, and *Uvariodendron usambarense*. The plants potential for future antiplasmodial investigations are *D. capricornuta* and *G. suaveolens* subs. *usambaricum*.

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