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RESEARCH ARTICLE

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In vitro antiprotozoan activity and mechanisms of action of selected Ghanaian medicinal plants against *Trypanosoma*, *Leishmania*, and *Plasmodium* parasites

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Trypanosomiasis, leishmaniasis, and malaria are protozoan infections of public health importance with thousands of new cases recorded annually. Control of these infection(s) with existing chemotherapy is limited by drug toxicity, lengthy parenteral treatment, affordability, and/or the emergence of resistant strains. Medicinal plants on the other hand are used in the treatment of various infectious diseases although their chemical properties are not fully evaluated. In this study, we screened 112 crude extracts from 72 selected Ghanaian medicinal plants for anti-*Trypanosoma*, anti-*Leishmania*, and anti-*Plasmodium* activities in vitro and investigated their mechanisms of action. Twenty-three extracts from 20 plants showed significant antiprotozoan activity against at least 1 of 3 protozoan parasites screened with IC_{50} values less than 20 μg/ml. Eleven extracts showed high anti-*Trypanosoma* activity with *Bidens pilosa* whole plant and *Morinda lucida* leaf extracts recording the highest activities. Their IC_{50} (selectivity index [SI]) values were 5.51 μg/ml (35.00) and 5.96 μg/ml (13.09), respectively. Nine extracts had high anti-*Leishmania* activity with *Annona senegalensis* and *Cassia alata* leaf extracts as the most active. Their IC_{50} (SI) values were 10.8 μg/ml (1.50) and 10.1 μg/ml (0.37), respectively. Six extracts had high anti-*Plasmodium* activity with the leaf and stem-bark extracts of *Terminalia ivorensis* recording the highest activity. Their IC_{50} (SI) values were 7.26 μg/ml (129.36) and 17.45 μg/ml (17.17), respectively. Only *M. lucida* at 25 μg/ml induced significant apoptosis-like cell death in *Trypanosoma* parasites. Anti-*Leishmania*

Abbreviations: FACS, fluorescent activated cell sorting

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active extracts induced varying morphological changes in *Leishmania* parasites such as multiple nuclei and/or kinetoplast, incomplete flagella division, or nuclear fragmentation. Active extracts may be potential sources for developing new chemotherapy against these infections.

KEYWORDS

apoptosis, in vitro screening, *Leishmania donovani*, medicinal plants, morphology, *Plasmodium falciparum*, *Trypanosoma brucei brucei*

1 | INTRODUCTION

Protozoan infections are a major health problem causing significant morbidity and mortality in Africa, Asia, and Latin America (World Health Organization [WHO], 2015b). Although chemotherapy is one of the main forms of controlling protozoan infection, it is limited by the accessibility, adverse side effects, and the emergence of resistant parasites to available drugs.

African trypanosomiasis and leishmaniasis are protozoan infections caused by kinetoplastids. They are considered as the main pathogens of neglected tropical diseases. Trypanosomiasis is caused by *Trypanosoma brucei* species, transmitted by the tsetse fly, and threatens the lives of 50 million people in over 36 countries in sub-Saharan Africa with an estimated 30,000 new cases each year (Adeyemi, Sykes, Akanji, & Avery, 2011; Centers for Disease Control and Prevention, 2016; WHO, 2016a, 2016b). Chronic and acute forms of human African trypanosomiasis are caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*, respectively, whereas *Trypanosoma brucei brucei* causes Nagana in animals (Shi, Wei, Pan, & Tabel, 2006). Leishmaniasis on the other hand is caused by over 20 different species of *Leishmania* and is transmitted by the sand fly. Approximately 350 million people are at risk of infection in over 88 countries across the world (Bensoussan, Nasereddin, Jonas, Schnur, & Jaffe, 2006). An estimated 1.3 million new cases and 20,000 to 30,000 deaths occur annually (WHO, 2016a). There are three forms of leishmaniasis: cutaneous, mucocutaneous, and visceral leishmaniasis (Alvar, Yactayo, & Bern, 2006; Desjeux, 2004; Herwaldt, 1999; Lysenko, 1971; Murray, Berman, Davies, & Saravia, 2005). Last but not least, malaria, a typical protozoan infection that affects millions of people worldwide, is caused by *Plasmodium* species and transmitted by the female *Anopheles* mosquito. Sub-Saharan Africa alone accounts for 89% of malaria cases with 78% of malaria fatality occurring in children under 5 years old (WHO, 2015a).

The control of all three protozoan infections is limited by drug toxicity, resistant strains of parasites, and economic/financial factors. In the case of malaria for example, resistant malaria is a very serious problem worldwide. Quinine was the drug of choice for close to 100 years before it was replaced with artemisinin isolated from *Artemisia annua* due to the emergence of resistant parasites. There are however reports of artemisinin failure in South East Asia making it necessary to develop new effective chemotherapy (Adeyemi et al., 2011; Bacchi, 2009; Balunas & Kinghorn, 2005; Singh & Sivakumar, 2004). Despite the use of medicinal plants in the treatment of ailments including those caused by protozoan pathogens in Africa (Ankrah et al., 2003; Barrett, Boykin, Brun, & Tidwell, 2007; Okpekon et al., 2004; Rahmatullah et al., 2010; Trouiller et al., 2002), scientific evidence of the medicinal properties of these plants have not been fully evaluated (Abu & Uchendu, 2011; Fathuddin, 2011; M. A. Ibrahim et al., 2010; N. Nweze, Anene, & Asuzu, 2011; Nweze, 2012; Ogbadoyi, Kabiru, & Omotosho, 2011; Wurochekke & Anyanwu, 2012).

This study therefore aimed at screening selected Ghanaian medicinal plants, based on knowledge of their traditional use in treating various infections/diseases, for anti-*Trypanosoma*, anti-*Leishmania*, and anti-*Plasmodium* properties in vitro.

2 | MATERIALS AND METHODS

2.1 | Plant materials and preparation of crude extracts

Based on the traditional knowledge of their medicinal use, extracts from different parts (leaves, stem bark, fruits, seeds, or roots) of 72 plants were collected in Ghana by the Centre for Plant Medicine Research, Mampong, Ghana, during the period of October 2010 to November 2012. Authentication was done by one of the authors (Y. S.). Voucher specimens have been deposited in Centre for Plant Medicine Research. The air-dried and pulverized plant samples (200 g) were extracted by 50% aqueous EtOH 3 times under room temperature. The accumulated solution was evaporated in a rotary evaporator at 40 °C to obtain the crude extract. The extracts were kept in sterile tubes and stored at 4 °C until use. Prior to drug-sensitivity assays, 100-mg/ml stock solutions were prepared with 50% EtOH and filter sterilized.

2.2 | In vitro culture of parasitic protozoans

GUTat 3.1 strain of the bloodstream form of *T. b. brucei* was used for this study. Parasites were cultured in vitro according to conditions established previously (Yabu et al., 1998). Parasites were used for assays when they reached a concentration of 1×10^6 parasites/ml. Estimation of parasitemia was done with the Neubauer's counting chamber. Parasites were diluted to a concentration of 3×10^5 parasites/ml with HM1-9 medium and used for the various experiments. For *Leishmania* parasites, promastigote forms of *Leishmania donovani* (MHOM/NP/03/D10) cultures were used in this study with the culture media previously established with slight modifications

(Mottram, 2008). The parasites were used for assays when they reached a concentration of 1×10^7 parasites/ml. Parasitemia was estimated with the Neubauer's counting chamber. Parasites were diluted to a concentration of 2.5×10^6 parasites/ml with M199 medium for drug assays.

For the maintenance of malaria cultures, 3D7 strain of *Plasmodium falciparum* was cultured based on previously established protocols with modifications (Trager & Jensen, 1976). Culture media were changed daily, and the level of parasitemia was determined by counting red blood cells (RBCs) on a Giemsa-stained thin blood smear under a light microscope. *Plasmodium* culture, at 5% parasitemia, was synchronized with 5% sorbitol to obtain ring stage, trophozoite parasites, and incubated for an extra 48 hr to obtain trophozoites, which were used in the screening of plant extracts.

2.3 | In vitro antiparasitic screening assays of plant extracts

2.3.1 | Antikinetoplastid activity

The Alamar Blue assay (Alamar Blue®, Life Technologies™, USA) was used to determine the antitrypanosomal and antileishmanial activities of plant extracts. Assays were carried out in a 96-well plate following manufacturer's instructions with modifications (Kwofie et al., 2016). Either 1.5×10^4 *Trypanosoma* parasites per well or 1.25×10^5 *Leishmania* parasites per well were seeded with varied concentrations of crude extracts ranging from 0 to 200 μg/ml. Final concentration of EtOH was kept under 1%, and a solvent control (negative control) was used in all assays. Berberine and amphotericin B were used as positive controls for *Trypanosoma* and *Leishmania*, respectively. After a 24-hr incubation of *Trypanosoma* and 44-hr incubation of *Leishmania* parasites with or without plant extracts, 10% Alamar Blue dye was added and incubated for 24 and 4 hr in darkness, respectively. After 48 hr, the plate was read for absorbance at a wavelength of 540 nm (reference wavelength of 595 nm) using a spectrophotometer (TECAN Sunrise Wako, Japan). Trend curves were drawn to obtain IC_{50} values of plant extracts.

2.3.2 | Anti-*Plasmodium* activity

Fluorescent activated cell sorting (FACS) was used to determine the anti-*Plasmodium* activity of the plant extracts. Synchronized parasites at a packed volume of 2% hematocrit and 1% parasitemia were challenged with 0- to 25-μg/ml plant extracts for 48 hr. Artesunate (Sigma-Aldrich, USA) was used as the positive control, whereas RBCs at 2% hematocrit only and packed RBCs plus 2.5% EtOH served as negative and vehicle controls, respectively. SYBR Green I solution (0.25 μl of 10,000 × SYBR Green I/1 ml of 1 × phosphate buffered saline [PBS]) was added to each well after the 48-hr incubation period and incubated for additional 30 min in the dark at 37 °C. Plates were read using the Guava EasyCyte 5HT FACS machine (Millipore, USA) following the manufacturer's instructions.

2.4 | In vitro cytotoxicity assay

Jurkat cells (human acute T-cell leukemia cells) were obtained from the RIKEN BioResource Centre Cell Bank (Japan) and maintained in RPMI supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin–L-glutamine. The cells were incubated at 37 °C under 5% CO_2 in fully humidified conditions. The toxicity of plant extracts against the Jurkat cells was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were plated at a density of 3.0×10^5 cells/ml into a 96-well plate. Cells were treated with various concentrations of each of the plant extracts and incubated for 48 hr. MTT solution was added to each well, and the cells were incubated for an extra 4 hr. The precipitated MTT-formazan product was dissolved in 0.04 N HCl–isopropanol, and the amount of formazan was measured at a wavelength of 570 nm by a microplate spectrophotometer (Tecan Infinite M200 Pro, Austria). Cytotoxicity was calculated as the percentage of life cells relative to the control culture. The selectivity index (SI) was expressed as the ratio of the IC_{50} value obtained for mammalian cells to the IC_{50} values obtained for parasites (Kwofie et al., 2016).

2.5 | Apoptosis assay

Nexin assay using EasyCyte 5HT FACS machine (Millipore, USA) was performed to investigate apoptotic properties of active crude extracts against *T. brucei*. Seeding and incubation of parasites with crude extracts were done under the same conditions of in vitro antiparasitic screening assay as described above. After 24 hr incubation, 10% Nexin reagent (Millipore, USA) was added to the *Trypanosome* culture and then subjected to FACS analysis (Guava EasyCyte 5HT, Millipore, USA) following the manufacture's instruction (Kwofie et al., 2016).

2.6 | Effect of plant crude extract on *Leishmania* parasite morphology

To investigate the effect crude extracts with strong anti-*Leishmania* activity, IC_{50} less than 20 μg/ml had on parasite morphology, fluorescence microscopy was performed with 4′,6-diamidino-2-phenylindole (DAPI). Parasites were incubated with or without each anti-*Leishmania* active extract at a concentration twice the IC_{50} value for 24 hr. *Leishmania* parasites were harvested and fixed with 70% EtOH on eight well chamber slides at −20 °C for 1 hr. After washing twice with PBS for 5 min each and 0.1% Triton X-100 in PBS for 15 min at room temperature, parasite nucleus and kinetoplasts were stained with DAPI (5 μg/ml in PBS) for 10 min. Slides were then washed as described above, mounted with a mounting reagent and covered with cover slips. The slides were observed under the fluorescent microscope (Olympus, BX-530, Japan; Kwofie et al., 2016).

3 | RESULTS

3.1 | In vitro antiparasitic activity

Plants and plant parts used in this study are indicated in Table 1. One hundred twelve plant extracts representing 72 plant species belonging to 38 families were selected according to traditional knowledge designated as activities in Table 1. Table 1 also contains the botanical names, families, and parts of the plants that were screened. Forty-three (38.4%) of the extracts were obtained from leaves, 33 (29.5%) from stem barks, 14 (12.5%) from roots, and 9 (8.03%) from the whole

TABLE 1 List of selected Ghanaian medicinal plants and their known activities compiled by Centre for Plant Medicine Research

Plant species	Family	Plant part	Components	Activities
Acacia nilotica	Fabaceae	Stem bark	Tannins, alkaloids, saponins	Anti-*Trypanosoma*, anti-*Plasmodium*
Acanthospermum hispidum	Asteraceae	Whole plant	Essential oil, alkaloids	Antiviral, anti-*Plasmodium*, anti-herpesvirus, anti-pseudorabies virus
Aframomum melegueta	Zingiberaceae	Seeds	Tannins, saponin, flavonoids, steroid	Anti-HIV, antimicrobial
Afzelia africana	Fabaceae	Stem bark	Alkaloids, tannins, flavonoids, saponins	Anti-*Trypanosoma*, anti-*Plasmodium*, antibacterial
Alchornea cordifolia	Euphorbiaceae	Leaves	Yohimbine, tannins, saponins, alkaloids	Anti-HIV-1 (seed), anti-*Trypanosoma cruzi* (leaf)
Alstonia boonei	Apocynaceae	Leaves	Indolealkaloids, triterpenoids, tannins	Anti-*Plasmodium*
Alstonia boonei	Apocynaceae	Stem bark	Indolealkaloids, triterpenoids, tannins	Anti-*Plasmodium*
Annona senegalensis	Annonaceae	Leaves	Alkaloids, flavonoids, tannins, terpenoids, saponins	Anti-*Trypanosoma*
Annona senegalensis	Annonaceae	Stem cutting	Flavonoids, tannins, alkaloids, saponins, glycosides	Anti-*Trypanosoma*
Anogeissus schimperi	Combretaceae	Leaves	Tannins, polysaccharide	Anti-*Plasmodium*, antihelminth
Anogeissus schimperi	Combretaceae	Stem bark	Tannins, polysaccharide	Anti-*Plasmodium*, antihelminth
Anogeissus schimperi	Combretaceae	Root	Tannins, polysaccharide	Anti-*Plasmodium*, antihelminth
Anthocleista nobilis	Loganiaceae	Leaves	Glycosides, saponin, steroid	Antihelminth, analgesic, antipyretic
Anthocleista nobilis	Loganiaceae	Stem bark	Quinolone, alkaloid, monoterpene, glycoside	Antimicrobial, anti-inflammatory
Anthocleista nobilis	Loganiaceae	Roots	Anthocleistol	Anti-*Leishmania*, hypoglycemic
Balanites aegyptiaca	Balanitaceae	Stem bark	Tannin, saponin,	Anti-*Plasmodium*
Baphia nitida	Fabaceae	Stem bark	Tannins, flavonoids, saponin glycosides	Antiparasitic skin disease
Bidens pilosa	Asteraceae	Whole plant	Essential oils, flavonoids, alkaloids, saponins, triterpenes	Anti-*Plasmodium*
Bridelia ferruginea	Euphorbiaceae	Leaves	Flavonoids, triterpenoids, tannins	Antiviral, anti-*Plasmodium*, anti-*Trypanosoma*
Calotropis procera	Asclepiadaceae	Leaves	Saponin, tannin, alkaloids	Anti-HIV
Carapa procera	Meliaceae	Stem bark	Flavonoids, glycoside, tannins, saponin	Anti-HIV
Carica papaya	Caricaceae	Seeds	Coumarins, alkaloids, flavonoids	Anti-*Plasmodium*, anti-Entamoeba antiparasitic
Cassia alata	Fabaceae	Leaves	Flavonoids, glycosides	Analgesic, antihyperglycemic
Cassia siamea	Fabaceae	Stem bark	Anthraquinones, flavonoids	Anti-*Plasmodium*
Cassia sieberiana	Fabaceae	Roots	Galactosides, flavonoids	Anti-*Trypanosoma*, antiulceragentic
Cassia sieberiana	Fabaceae	Leaves	Flavonoids, alkaloids	Anti-*Trypanosoma*
Cassia podocarpa	Fabaceae	Leaves	Anthraquinone	Anti-*Plasmodium*
Cassia occidentalis	Fabaceae	Seeds	Anthraquinone, flavonoids	Antiparasitic, anti-HIV
Cassia occidentalis	Fabaceae	Leaves	Anthraquinone, flavonoids	Antiparasitic, anti-HIV
Cassia occidentalis	Fabaceae	Whole plant	Anthraquinone, flavonoids	Antiparasitic, anti-HIV
Ceiba pentandra	Bombacaceae	Stem bark	Isoflavones, sesquiterpenoid	Antiparasitic
Cinnamomum zeylanicum	Lauraceae	Leaves	Essential oils, alkaloids, tannins, triterpenoids, coumarins	Antiviral, clinical trial for AIDS patients
Cinnamomum zeylanicum	Lauraceae	Stem bark	Essential oils, alkaloids, tannins, triterpenoids, coumarins	Antiviral, clinical trial for AIDS patients
Citrus aurantifolia	Rutaceae	Leaves	Flavonoids, carotenoids	Anti-HIV, anti-*Plasmodium*
Citrus aurantifolia	Rutaceae	Fruits	Flavonoids, terpenes	Anti-HIV, anti-*Plasmodium*, antiscurvy
Clausena anisata	Rutaceae	Roots	Essential oil, indolealkaloids, coumarins	Anti-HIV-1 and HIV-2 (H_2O ext.)
Cleistopholis patens	Annonaceae	Leaves	Glycosides, terpenoids	Anti-*Trypanosoma*, anti-*Plasmodium*, antihelminth
Cleistopholis patens	Annonaceae	Stem bark	Flavonoids, saponins, alkaloids	Anti-*Trypanosoma*
Cola cordifolia	Sterculiaceae	Stem bark	Tannin, phenols	Anti-*Trypanosoma*
Cola cordifolia	Sterculiaceae	Leaves	Tannin, phenols	Anti-*Trypanosoma*
Cola acuminata	Sterculiaceae	Leaves and stem bark	Purine alkaloid, catechin, (tannin)	Antipyrogenic, diarrhea treatment

(Continues)

TABLE 1 (Continued)

Plant species	Family	Plant part	Components	Activities
Cymbopogon citratus	Poaceae	Whole plant	Essential oils, alkaloids, saponins, tannins, flavonoids	Anti-*Plasmodium*, anti-*Leishmania*
Eugenia species	Myrtaceae	Seed	Essential oil, flavonoid, tannins,	Antifungal, antibacterial, anti-inflammatory
Ficus capensis	Moraceae	Stem bark	Phenols, tannins, alkaloid	Anti-*Trypanosoma*, antibacterial
Ficus capensis	Moraceae	Leaves	Saponins, flavonoids, glucosides	Anti-*Trypanosoma*, anti-*Plasmodium*, antidiarrhea
Garcinia kola	Guttiferae	Leaves	Tannins, triterpenoids, flavonoids, coumarins	Anti-*Plasmodium*
Garcinia kola	Guttiferae	Stem bark	Tannins, triterpenoids, flavonoids, coumarins	Anti-*Plasmodium*
Glyphaea brevis	Tiliaceae	Leaves	Tannins, alkaloids, flavonoids	Anti-*Trypanosoma*
Gossypium arboreum	Malvaceae	Leaves	Flavonoids, steroids, tannins	Anti-HIV
Heliotropium indicum	Boraginaceae	Whole plant	Pirrolizidine alkaloid, hydrolysable tannin	Antiviral
Khaya senegalensis	Meliaceae	Stem bark	Tannins, saponin, glycoside	Anti-*Plasmodium*, antihelminth
Khaya grandifoliola	Meliaceae	Stem bark	Alkaloids, saponins, tannins	Anti-*Plasmodium*, antimicrobial
Lantana camara	Verbenaceae	Whole plant	Triterpenoids, flavonoids	Anti-*Plasmodium*, anti-*Leishmania*
Lippia multiflora	Verbenaceae	Leaves	Essential oil, flavonoid, saponin	Anti-*Trypanosoma*, anti-*Leishmania*
Lippia multiflora	Verbenaceae	Roots	Essential oil, flavonoid, saponin	Anti-*Plasmodium*
Lophira lanceolata	Ochnaceae	Stem bark	Flavonoid, resin, saponin, alkaloid	Anti-*Trypanosoma*, anti-*Plasmodium*
Lophira lanceolata	Ochnaceae	Roots	Alkaloids	Anti-*Trypanosoma*
Mangifera indica	Anarcadiaceae	Stem bark	Tannins, flavonoids, triterpenoids	Anti-*Trypanosoma*
Mangifera indica	Anarcadiaceae	Leaves	Tannins, flavonoids, triterpenoids	Anti-*Trypanosoma*
Mentha piperita	Lamiaceae	Leaves	Essential oil, triterpenes, flavonoids	Anti-*Trypanosoma*
Mitragyna inermis	Rubiaceae	Leaves	Indole alkaloids, triterpenoids	Anti-*Trypanosoma*
Mitragyna inermis	Rubiaceae	Stem bark	Indole alkaloids, triterpenoids	Anti-*Trypanosoma*
Mondia whitei	Asclepiadaceae	Root	Glycoside, resin, glucose	Anti-*Schistosoma*, antipyretic
Morinda lucida	Rubiaceae	Leaves	Anthraquinones, iridoids, tannins	Anti-*Trypanosoma*, anti-*Plasmodium*
Morinda lucida	Rubiaceae	Roots	Anthraquinones, iridoids, tannins	Anti-*Trypanosoma*, anti-*Plasmodium*
Morinda lucida	Rubiaceae	Stem bark	Anthraquinones, iridoids, tannins	Anti-*Trypanosoma*, anti-*Plasmodium*, anti-*Leishmania*
Moringa oleifera	Moringaceae	Leaves	Glycoside, saponin	Anti-HIV
Nauclea latifolium	Rubiaceae	Stem bark	Indoloquinolizidine alkaloids, tannins	Anti-*Plasmodium*
Nauclea latifolium	Rubiaceae	Roots	Indoloquinolizidine alkaloids, tannins	Anti-*Plasmodium*
Nauclea latifolium	Rubiaceae	Leaves	Indoloquinolizidine alkaloids, tannins	Anti-*Plasmodium*
Newbouldia laevis	Bignoniaceae	Leaves	Phenylethanoid glycoside, apigenin, alkaloid	Anti-*Plasmodium*, antiparasitic, antihelminth
Newbouldia laevis	Bignoniaceae	Stem bark	Phenylethanoid glycoside, apigenin, alkaloid	Anti-*Plasmodium*, antiparasitic, antihelminth
Ocimum gratissimum	Lamiaceae	Whole plant	Essential oil (eugenol), tannins	Anti-HIV-1 and HIV-2, anti-*Plasmodium*
Parkia clappertoniana	Fabaceae	Leaves	Saponin, flavonoid, tannins	Antiviral, anti-HIV, antidiarrhea
Parkia clappertoniana	Fabaceae	Stem bark	Saponin, steroid, triterpenes	Anti-HIV
Paullinia pinnata	Sapindaceae	Roots	Triterpene saponins, tannins, flavonoid glycosides	Anti-*Plasmodium*, antibacterial, antioxidant
Picralima nitida	Apocynaceae	Leaves	Indole alkaloids	Anti-*Plasmodium*, anti-*Trypanosoma*
Picralima nitida	Apocynaceae	Stem bark	Indole alkaloids	Anti-*Plasmodium*, anti-*Trypanosoma*
Piliostigma thonningii	Fabaceae	Leaves	Tannins, alkaloids, flavonoids	Antihelminth
Piliostigma thonningii	Fabaceae	Stem cutting	Tannins, alkaloids, flavonoids	Antihelminth
Piper guineense	Piperaceae	Leaves	Essential oil, pipelines, lignin	Antiviral
Piper guineense	Piperaceae	Seed	Essential oil, pipelines, lignin	Antiviral
Pseudocedrela kotschyi	Meliaceae	Stem bark	Tannin, saponin, limonoid, sesquiterpenoid	Anti-*Leishmania*, anti-*Trypanosoma*, anti-*Plasmodium*
Pseudocedrela kotschyi	Meliaceae	Root bark	Tannin, saponin, limonoid, sesquiterpenoid	Anti-*Leishmania*, anti-*Trypanosoma*, anti-*Plasmodium*

(Continues)

TABLE 1 (Continued)

Plant species	Family	Plant part	Components	Activities
Psidium guajava	Myrtaceae	Leaves	Tannins, essential oil, triterpenoids, flavonoids	Antimicrobial (all strains), anti-*Plasmodium*, anti-*Leishmania*
Pterocarpus santalinoides	Fabaceae	Stem bark	Alkaloids, flavonoids, tannins	Anti-HIV, antimicrobial
Pycnanthus angolensis	Myristicaceae	Leaves	Isoflavone, pycnanthuquinone	Anti-*Plasmodium*
Pycnanthus angolensis	Myristicaceae	Stem bark	Isoflavone, pycnanthuquinone	Anti-*Plasmodium*
Securidaca longepedunculata	Polygalaceae	Leaves	Saponins, tannins, cardiac glycoside, steroid	Anti-*Trypanosoma*
Solanum torvum	Solanaceae	Leaves	Steroidal sapogenins, steroidal alkaloids, isoflavonoid, steroidal glycosides	Antiviral, anti-*Plasmodium*
Solanum torvum	Solanaceae	Stem bark	Steroidal sapogenins, steroidal alkaloids, isoflavonoid, steroidal glycosides	Antiviral, anti-*Plasmodium*
Sorghum bicolor	Poaceae	Leafstalk	Alkaloids, saponins, tannins	Anti-HSV, antiviral
Spondias mombin	Anacardiaceae	Leaves	Not available	Antiviral
Tabernaemontana crassa	Apocynaceae	Leaves	Tannins, saponins, alkaloids	Antihelminth, antipyregic
Tabernaemontana crassa	Apocynaceae	Root	Tannins, saponins, alkaloids	Antipyretic, anti-snake venom
Tamarindus indica	Fabaceae	Stem bark	Saponins, tannins, glycoside	Anti-*Trypanosoma*
Tamarindus indica	Fabaceae	Leaves	Phenols, flavonoid	Anti-*Trypanosoma*
Terminalia ivorensis	Combretaceae	Stem bark and leaves	Terminolic acid, quercetin, β-glycyrrhetinic acid	Anti-*Trypanosoma*, anti-*Plasmodium*
Terminalia ivorensis	Combretaceae	Leaves	Terminolic acid, quercetin, β-glycyrrhetinic acid	Anti-*Trypanosoma*
Theobroma cacao	Serculiaceae	Leaves	Purine alkaloids, tannins, flavonoids	Anti-HIV
Theobroma cacao	Serculiaceae	Roots	Purine alkaloids, tannins, flavonoids	Anti-HIV
Theobroma cacao	Serculiaceae	Stem bark	Purine alkaloids, tannins, flavonoids	Anti-HIV
Thonningia sanguinea	Balanophoraceae	Whole plant	Alkaloids, tannins, flavonoids	Anti-*Plasmodium*, antifungal, antimicrobial
Treculia africana	Moraceae	Stem bark	Catechin, cyaniding glycoside	
Tridax procumbens	Asteraceae	Whole plant	Flavonoids, alkyl esters, sterols	Anti-HIV
Vitex fosteri	Verbenaceae	Stem bark	Essential oils, flavonoids	Anti-*Trypanosoma*
Vitex fosteri	Verbenaceae	Leaves	Essential oils, flavonoids	Anti-*Trypanosoma*
Ximenia americana	Olacaceae	Stem and twigs	Tannins, flavonoids, alkaloids	Anti-*Trypanosoma*
Ximenia americana	Olacaceae	Leaves	Tannins, flavonoids, glycosides	Anti-*Trypanosoma*, antimicrobial
Zanthoxylum zanthoxyloides	Rutaceae	Root	Alkaloids (berberine), tannins, flavonoids, essential oil	Anti-*Leishmania*
Zanthoxylum zanthoxyloides	Rutaceae	Stem bark	Alkaloids (berberine), tannins, flavonoids, essential oil	Anti-*Plasmodium*
Zanthoxylum zanthoxyloides	Rutaceae	Leaves	Alkaloids (berberine), tannins, flavonoids, essential oil	Antiparasitic

plant. Together, 13 (11.6%) extracts were prepared from seeds, fruits, stem cuttings, leafstalk, and twigs (Table 1).

Antikinetoplastid and anti-*Plasmodium* properties of extracts were investigated after a 48-hr incubation period. The IC_{50} values of the crude extracts against bloodstream *T. b. brucei* (GUTat 3.1), promastigote *L. donovani* (MHOM/NP/03/D10), and *P. falciparum* (3D7) strains are summarized in Table 2. Out of 112 crude extracts screened, 61, 41, and 13 were found to have varying degrees of activity against *T. b. brucei*, *L. donovani*, and *P. falciparum*, respectively. Eleven (9.82%) extracts had strong anti-*Trypanosoma* activity with IC_{50} values less than 20 μg/ml, whereas 30 (26.79%) and 20 (17.86%) extracts had moderate to fair activity with IC_{50} values in the range of 21–50 and 51–100 μg/ml, respectively. Nine (8.04%) extracts had high anti-*Leishmania* activity with IC_{50} values less than 20 μg/ml, whereas 22 (19.64%) and 10 (8.93%) extracts had moderate to fair activity with IC_{50} values ranging from 20 to 50 and 51 to 100 μg/ml, respectively. Six (5.36%) extracts had high anti-*Plasmodium* activity with IC_{50} values less than 20 μg/ml, whereas four (3.57%) and five (4.46%) extracts had moderate to fair activity with IC_{50} values ranging from 20 to 50 and 51 to 100 μg/ml, respectively. The IC_{50} values of the positive controls were 7.84, 0.1, and 0.01 μg/ml for berberine, amphotericin B, and artesunate, respectively.

The SI values for individual pathogens obtained using Jurkat cells are outlined in Table 2. The SI values of all but one crude extracts with strong anti-*Trypanosoma* activity, IC_{50} less than 20 μg/ml, were above 10.00. *Acanthospermum hispidum* whole plant extract was the only

TABLE 2 In vitro antiparasitic activity of screened crude extracts against *Trypanosoma*, *Leishmania*, and *Plasmodium* species, with cytotoxicity and SI values

		IC_{50} (μg/ml)				Selectivity index (SI)		
Plant species	**Plant part**	**Jurkat**	***T. b. brucei***	***L. donovani***	***P. falciparum***	***T. b. brucei***	***L. donovani***	***P. falciparum***
Acacia nilotica	Stem bark	39.59	79.32	>1,000	208.33	0.49	<0.04	0.17
Acanthospermum hispidum	Whole plant	55.50	**7.57**	32.1	>1,000	7.33	1.73	<0.06
Aframomum melegueta	Seed	62.49	168.83	>1,000	509.68	0.37	<0.06	0.12
Afzelia africana	Stem -bark	232.60	233.58	77.1	222.36	1.00	3.02	1.05
Alchornea cordifolia	Leaves	73.01	219.80	443.2	**17.44**	0.33	0.16	4.19
Alstonia boonei	Leaves	183.48	51.79	>1,000	>1,000	3.54	<0.18	<0.18
Alstonia boonei	Stem bark	736.36	25.96	>1,000	>1,000	28.37	<0.74	<0.74
Annona senegalensis	Leaves	273.49	182.23	**10.8**	>1,000	1.50	25.32	<0.27
Annona senegalensis	Stem cutting	127.95	353.89	27.8	>1,000	0.36	4.60	<0.13
Anogeissus schimperi	Leaves	51.72	34.44	>1,000	**18.32**	1.50	<0.05	2.82
Anogeissus schimperi	Stem bark	38.29	>1,000	>1,000	25.86	<0.04	<0.04	1.48
Anogeissus schimperi	Root	42.15	105.18	>1,000	50.46	0.40	<0.04	2.08
Anthocleista nobilis	Leaves	245.59	24.68	41.5	>1,000	9.95	5.92	<0.25
Anthocleista nobilis	Stem bark	761.33	410.39	843.7	>1,000	1.86	0.90	<0.76
Anthocleista nobilis	Roots	716.41	33.32	79.0	>1,000	21.50	9.07	<0.72
Balanites aegyptiaca	Stem bark	804.91	>1,000	173.6	>1,000	<0.80	4.64	<0.80
Baphia nitida	Stem bark	990.55	>1,000	34.4	>1,000	<0.99	28.80	<0.99
Bidens pilosa	Whole plant	192.85	**5.51**	28.9	>1,000	35.00	6.67	<0.19
Bridelia ferruginea	Leaves	392.80	43.28	**16.5**	83.06	9.08	23.81	4.73
Calotropis procera	Leaves	39.64	30.34	>1,000	>1,000	1.31	<0.04	<0.04
Carapa procera	Stem bark	85.17	117.94	>1,000	>1,000	0.72	<0.09	0.09
Carica papaya	Seed	>1,000	>1,000	>1,000	519.38	1.0	1.0	>1.92
Cassia alata	Leaves	371.46	>1,000	**10.1**	57.60	<0.37	36.78	6.44
Cassia siamea	Stem bark	429.33	344.47	>1,000	>1,000	1.25	<0.43	<0.43
Cassia sieberiana	Roots	917.32	289.69	142.6	432.48	3.17	6.43	2.12
Cassia sieberiana	Leaves	48.48	45.87	62.9	>1,000	1.06	0.77	<0.05
Cassia podocarpa	Leaves	453.87	54.05	>1,000	>1,000	8.40	<0.45	<0.45
Cassia occidentalis	Seeds	926.63	>1,000	>1,000	>1,000	<0.93	<0.93	<0.93
Cassia occidentalis	Leaves	329.94	>1,000	>1,000	>1,000	<0.33	<0.33	<0.33
Cassia occidentalis	Whole plant	446.19	37.83	>1,000	>1,000	11.79	<0.45	<0.45
Ceiba pentandra	Stem bark	100.52	98.93	31.1	>1,000	1.02	3.23	<0.1
Cinnamomum zeylanicum	Leaves	273.98	50.89	>1,000	>1,000	5.38	<0.27	<0.27
Cinnamomum zeylanicum	Stem bark	53.80	25.89	>1,000	>1,000	2.07	<0.05	<0.05
Citrus aurantifolia	Leaves	520.06	217.31	542.9	>1,000	2.39	0.96	<0.52
Citrus aurantifolia	Fruits	33.62	29.81	>1,000	34.83	1.13	<0.03	0.97
Clausena anisata	Roots	293.14	29.50	**12.1**	487.56	9.94	24.23	0.60
Cleistopholis patens	Leaves	484.76	214.57	>1,000	>1,000	2.26	<0.48	<0.48
Cleistopholis patens	Stem bark	214.62	>1,000	60.2	>1,000	<0.21	3.57	<0.21
Cola cordifolia	Stem bark	465.61	37.41	25.1	>1,000	12.45	18.55	<0.47
Cola cordifolia	Leaves	465.61	**10.08**	**18.2**	730.34	46.19	25.58	0.64
Cola acuminata	Stem bark	156.99	47.44	47.8	279.84	3.31	3.28	0.56
Cymbopogon citratus	Whole plant	311.65	123.77	162.2	694.06	2.52	1.92	0.45
Eugenia species	Seed	94.41	**8.50**	26.6	208.97	11.11	3.55	0.45
Ficus capensis	Stem-bark	56.66	36.10	37.0	>1,000	1.57	1.53	<0.06
Ficus capensis	Leaves	258.00	159.62	88.9	>1000	1.62	2.90	<0.26
Garcinia kola	Leaves	343.41	34.47	673.1	>1,,000	9.96	0.51	<0.34
Garcinia kola	Stem bark	439.64	485.32	159.4	>1,000	0.91	2.76	<0.44

(Continues)

TABLE 2 (Continued)

Plant species	Plant part	IC_{50} (μg/ml)				Selectivity index (SI)		
		Jurkat	*T. b. brucei*	*L. donovani*	*P. falciparum*	*T. b. brucei*	*L. donovani*	*P. falciparum*
Glyphaea brevis	Leaves	962.15	141.92	43.4	>1,000	6.78	22.17	<0.96
Gossypium arboreum	Leaves	519.49	>1,000	>1,000	**16.10**	<0.52	<0.52	32.27
Heliotropium indicum	Whole plant	>1,000	>1,000	119.4	117.32	1.0	>8.38	8.52
Khaya senegalensis	Stem bark	70.59	23.81	>1,000	>1,000	2.96	<0.07	<0.07
Khaya grandifoliola	Stem bark	50.21	180.58	43.2	616.37	0.28	1.16	0.08
Lantana camara	Whole plant	176.58	115.02	>1,000	33.39	1.54	<0.18	5.29
Lippia multiflora	Leaves	249.86	83.59	>1,000	>1,000	2.99	<0.25	<0.25
Lippia multiflora	Roots	497.66	837.72	>1,000	>1,000	0.59	<0.50	<0.50
Lophira lanceolata	Stem bark	45.83	429.80	68.6	>1,000	0.11	0.67	<0.05
Lophira lanceolata	Roots	38.63	288.66	66.0	>1,000	0.14	0.59	<0.04
Mangifera indica	Stem bark	494.59	>1,000	>1,000	>1,000	<0.49	<0.49	<0.49
Mangifera indica	Leaves	55.04	77.37	>1,000	**14.25**	0.71	<0.06	3.86
Mentha piperita	Leaves	55.03	49.35	>1,000	566.88	1.11	<0.05	0.002
Mitragyna inermis	Leaves	193.21	397.50	21.9	>1,000	0.49	8.82	<0.19
Mitragyna inermis	Stem bark	424.52	362.02	28.0	>1,000	1.17	15.16	<0.42
Mondia whitei	Root	433.19	35.10	31.0	>1,000	12.34	13.97	<0.43
Morinda lucida	Leaves	78.07	**5.96**	>1,000	>1,000	13.09	<0.08	<0.08
Morinda lucida	Roots	177.69	499.67	>1,000	>1,000	0.36	<0.18	<0.18
Morinda lucida	Stem bark	939.12	28.04	>1,000	>1,000	33.49	<0.94	<0.94
Moringa oleifera	Leaves	409.40	84.43	>1,000	>1,000	4.85	<0.41	<0.41
Nauclea latifolium	Stem bark	544.29	549.78	784.0	>1,000	0.99	0.69	<0.54
Nauclea latifolium	Roots	649.37	>1,000	138.9	>1,000	<0.65	4.68	<0.65
Nauclea latifolium	Leaves	268.05	54.12	>1,000	>1,000	4.95	<0.27	<0.27
Newbouldia laevis	Leaves	171.05	**9.47**	>1,000	593.79	18.06	<0.17	0.29
Newbouldia laevis	Stem bark	171.05	147.66	>1,000	>1,000	1.16	<0.17	<0.17
Ocimum gratissimum	Whole plant	420.17	22.47	>1,000	>1,000	18.70	<0.42	0.42
Parkia clappertoniana	Leaves	114.73	58.30	**17.3**	501.23	1.97	6.63	0.23
Parkia clappertoniana	Stem bark	42.37	99.99	**17.6**	>1,000	0.42	2.41	<0.04
Paullinia pinnata	Roots	926.63	63.23	130.1	291.55	14.65	7.12	3.18
Picralima nitida	Leaves	247.16	44.08	631.0	>1,000	5.61	0.39	<0.25
Picralima nitida	Stem bark	583.53	268.68	>1,000	95.19	2.17	<0.58	6.13
Piliostigma thonningii	Leaves	95.83	78.37	>1,000	>1,000	1.22	<0.10	<0.1
Piliostigma thonningii	Stem cutting	156.09	135.25	>1,000	514.63	1.15	<0.16	0.3
Piper guineense	Leaves	389.63	23.09	>1,000	621.7	16.87	<0.39	0.62
Piper guineense	Seed	77.48	17.44	>1,000	266.7	4.44	<0.08	0.29
Pseudocedrela kotschyi	Stem Bark	48.26	58.80	>1,000	510.96	0.82	<0.05	0.09
Pseudocedrela kotschyi	Root bark	101.18	59.41	>1,000	414.01	1.70	<0.10	0.24
Psidium guajava	Leaves	136.72	>1,000	>1,000	>1,000	<0.14	<0.14	<0.14
Pterocarpus santalinoides	Stem bark	128.17	>1,000	>1,000	907.52	<0.13	<0.13	0.14
Pycnanthus angolensis	Leaves	71.65	38.39	>1,000	>1,000	1.87	<0.07	<0.07
Pycnanthus angolensis	Stem bark	218.66	**9.76**	150.0	>1,000	22.40	1.46	<0.22
Securidaca longepedunculata	Leaves	45.83	237.43	>1,000	114.39	0.19	<0.05	0.40
Solanum torvum	Leaves	38.63	>1000	137.0	50.53	<0.04	0.28	0.76
Solanum torvum	Stem bark	494.59	>1000	601.4	>1,000	<0.49	0.82	<0.49
Sorghum bicolor	Leafstalk	55.04	24.01	34.1	127.75	2.29	1.61	0.43
Spondias mombin	Leaves	55.03	77.04	81.5	46.66	0.71	0.68	1.18
Tabernaemontana crassa	Leaves	193.21	20.84	>1,000	>1,000	9.27	<0.19	0.19
Tabernaemontana crassa	Root	424.52	163.16	>1,000	>1,000	2.60	<0.42	0.42

(Continues)

TABLE 2 (Continued)

Plant species	Plant part	IC_{50} (μg/ml)				Selectivity index (SI)		
		Jurkat	*T. b. brucei*	*L. donovani*	*P. falciparum*	*T. b. brucei*	*L. donovani*	*P. falciparum*
Tamarindus indica	Stem bark	433.19	40.02	>1,000	>1,000	10.82	<0.43	0.43
Tamarindus indica	Leaves	78.07	39.33	58.12	>1,000	1.98	1.34	0.08
Terminalia ivorensis	Stem bark and leaves	177.69	17.45	23.2	**10.35**	10.18	7.66	17.17
Terminalia ivorensis	Leaves	939.12	11.28	24.9	**7.26**	83.26	37.72	129.36
Theobroma cacao	Leaves	409.40	58.37	>1,000	>1,000	7.01	<0.41	<0.41
Theobroma cacao	Roots	544.29	334.65	>1,000	>1,000	1.63	<0.54	<0.54
Theobroma cacao	Stem bark	649.37	65.95	>1,000	>1,000	9.85	<0.65	<0.65
Thonningia sanguinea	Whole plant	268.05	139.89	**18.6**	133.59	1.92	15.38	2.00
Treculia africana	Stem bark	171.05	53.24	44.8	614.95	3.21	3.84	0.28
Tridax procumbens	Whole plant	171.05	310.74	>1,000	>1,000	0.55	<0.17	<0.17
Vitex fosteri	Stem bark	420.17	146.75	49.8	>1,000	2.86	8.44	<0.42
Vitex fosteri	Leaves	114.73	42.44	72.4	>1,000	2.70	1.58	<0.11
Ximenia americana	Stem and twigs	42.37	85.54	36.1	>1,000	0.50	1.17	<0.42
Ximenia americana	Leaves	926.63	180.30	>1,000	176.26	5.14	<0.93	5.25
Zanthoxylum zanthoxyloides	Root	247.16	39.43	**13.5**	334.77	6.27	18.30	0.74
Zanthoxylum zanthoxyloides	Stem bark	583.53	**5.96**	45.2	112.15	97.91	12.91	5.20
Zanthoxylum zanthoxyloides	Leaves	95.83	27.73	>1,000	>1,000	3.46	<0.10	<0.96
Positive control		7.84	0.1	0.01				

Note. Antiprotozoan activities and cytotoxicity are represented by IC_{50} values obtained from Alamar Blue (*Trypanosoma brucei brucei* and *Leishmania donovani*), SYBR Green (*Plasmodium falciparum*), and MTT (Jurkat) assays, respectively. The IC_{50} values are averages of three independent assays run for each crude extract. SI was determined by dividing the IC_{50} values for the Jurkat cells by the IC_{50} values for each parasite tested. Berberine, amphotericin B, and artesunate were used as positive controls for anti-*Trypanosoma*, anti-*Leishmania*, and anti-*Plasmodium* activities, respectively. Bold emphasis are for extracts with high activity against parasites.

anti-*Trypanosoma* active extract with an SI value below 10, SI of 7.33. With respect to anti-*Leishmania* active extracts, seven out of nine had SI values above 10.00. *Parkia clappertoniana* leaf extract and *P. clappertoniana* stem bark extract were the two extracts with SI values below 10, SI of 6.63 and 0.4, respectively. Three out of six anti-*Plasmodium* active extracts, *Gossypium arboreum* leaf and stem bark extracts, and *Terminalia ivorensis* leaf extract showed SI values greater than 10.00.

3.2 | Apoptosis inducing properties

The Nexin assay was performed to determine the apoptosis inducing properties of the eight anti-*Trypanosoma* active extracts. Parasites were challenged with 25 μg/ml of the active extracts for 24 hr and then subjected to apoptosis analysis. *Morinda lucida* induced the highest level of apoptosis (69.8%) against *Trypanosoma* parasites whereas the other extracts showed no significant induction of apoptotic cells (Figure 1). No extract was observed to induce significant apoptosis-like cell death in *Leishmania* parasites and *Plasmodium* parasites using the Nexin assay and the mitochondrial membrane potential assays, respectively (data not shown).

3.3 | Morphological changes of *L. donovani* promastigotes induced by active crude extracts

The morphological changes of *Leishmania* parasites induced by active crude extracts were observed by fluorescence microscopy. Parasites were fixed and stained with DAPI after 24 hr incubation with each crude extract at a concentration 2 times the IC_{50} value. Parasites treated with *Annona senegalensis* extracts were observed to have no kinetoplasts with an intact nucleus. The *A. senegalensis* extracts also induced incomplete parasite division with only a single observable flagellum, resulting in the formation of paired daughter cells. *Cola cordifolia* induced nuclear fragmentation and multiple flagella in parasites without cell division. *Clausena anisata*-treated parasites were aggregated with some abnormal morphology. On the other hand, *Bridelia ferruginea* parasites were observed to have fragmented nuclei and linked to each other with very prominent flagella. *Zanthoxylum zanthoxyloides*-treated parasites had no significant change in the nucleus and kinetoplast. *Cassia alata*-treated parasites however induced an increase in the number of nuclei and kinetoplast with very severe aggregation of parasites, and most of the parasites showed a short stumpy-like form. Although there were short stumpy forms in *Z. zanthoxyloides*-treated parasites, the aggregations were not severe. Parasites treated with both *Z. zanthoxyloides* and *C. alata* did not have prominent flagella. *P. clappertoniana*-treated parasites were observed to have fragmented nuclei and aggregated into small groups. The parasites appeared round shaped and stumpy like without prominent flagella (Figure 2). Anti-*Trypanosoma* active extracts were however not observed to induce significant and/or varied morphological changes in *Trypanosoma* parasites as observed in *Leishmania* parasites (data not shown).

4 | DISCUSSION

Traditional knowledge stake holders (herbalists, farmers, and local indigenes) take advantage of the medicinal properties of plants and their

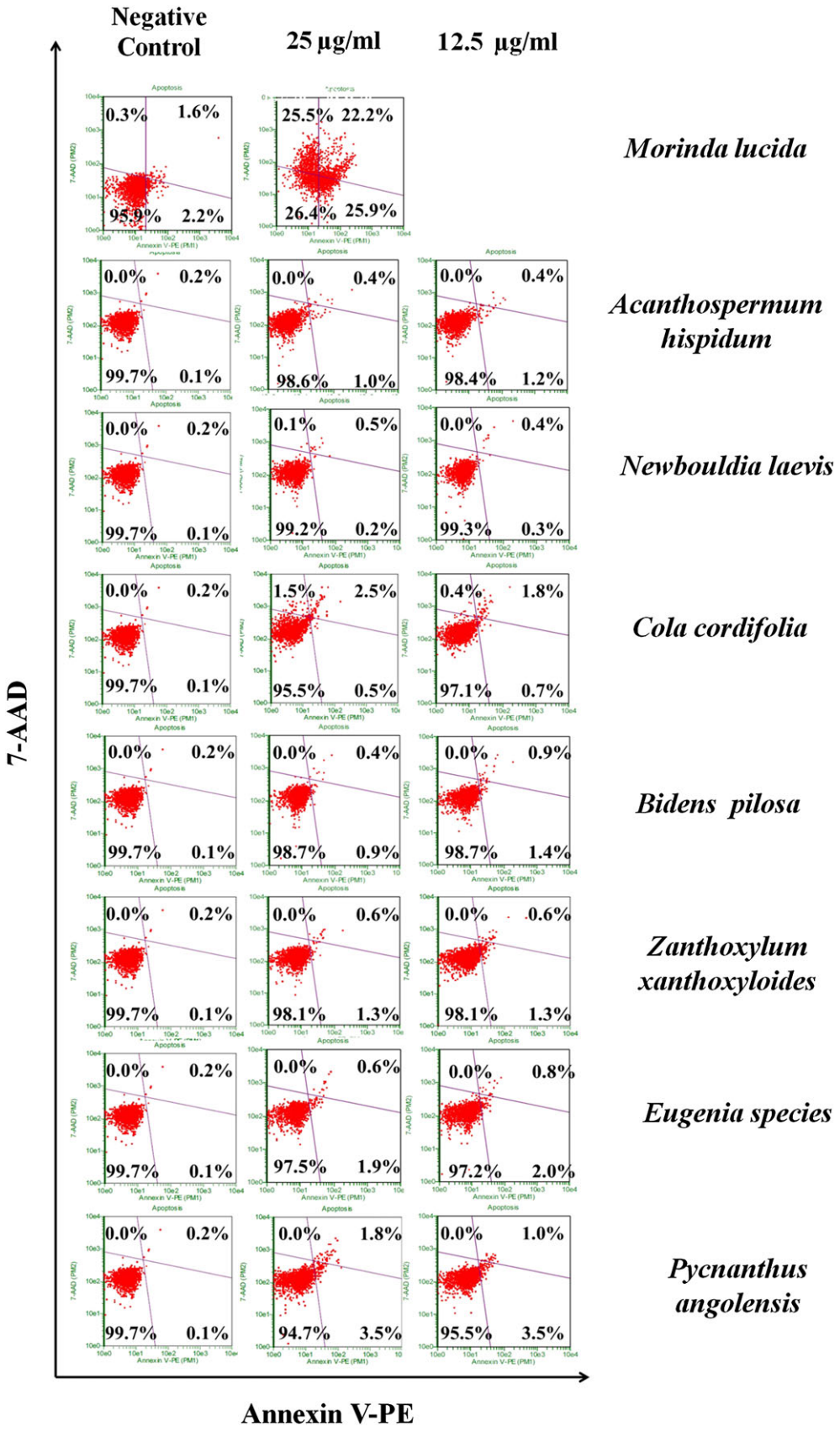

FIGURE 1 Signals of apoptosis induction in *Trypanosoma* parasites were detected based on the externalization of phosphatidylserine and DNA fragmentation. Data were obtained using the nexin assay and fluorescent activated cell sorting analysis. Results are represented by dot plots of four quadrants: Lower left = viable cells; lower right = early apoptotic cells; upper right = late apoptotic cells; upper left = necrotic cells [Colour figure can be viewed at wileyonlinelibrary.com]

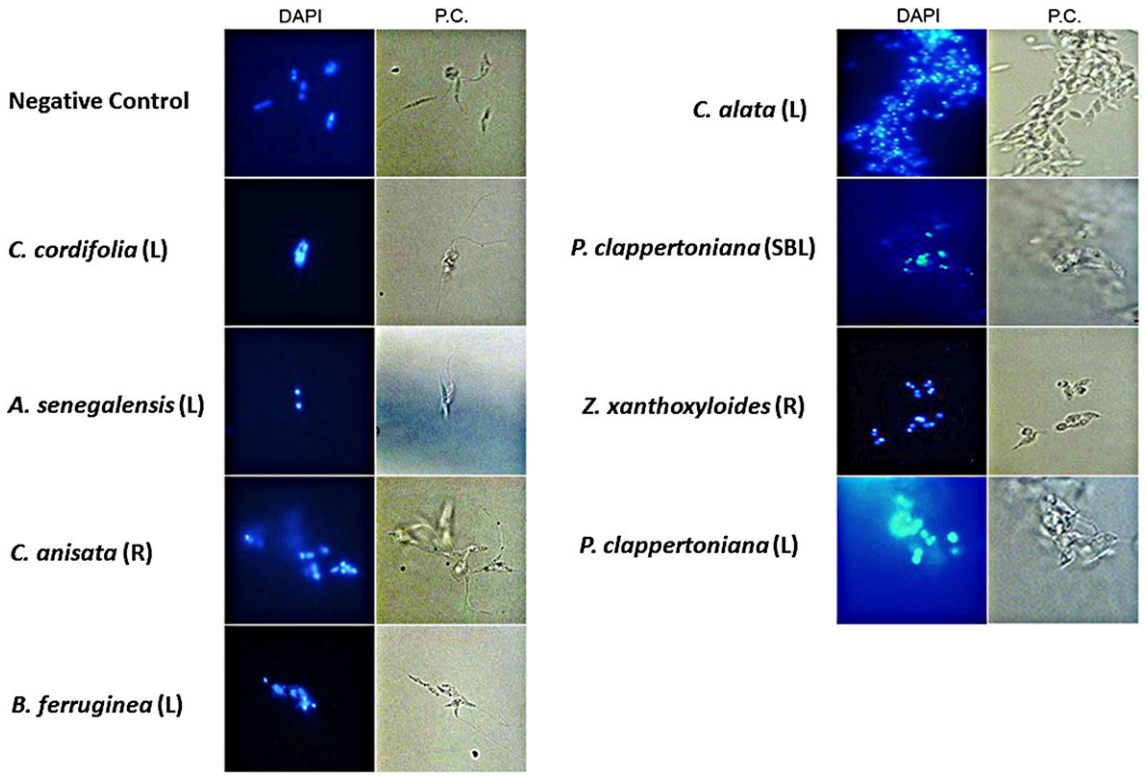

FIGURE 2 Fluorescence microscope view showing the effect of extract on the nucleus (N), kinetoplast (K), flagella (F), and morphology of the *Leishmania* parasite. Phase contrast (P.C.) is the normal view; parasite nucleus and kinetoplast were detected by 4′,6-diamidino-2-phenylindole (DAPI) [Colour figure can be viewed at wileyonlinelibrary.com]

usefulness in the treatment of a wide range of diseases including those caused by protozoans (Table 1). We previously reported that the component(s) of a plant varied between plant parts. *Coleus forskolii*, for example, contains forskolin, an activator of cyclic AMP, only in some specific parts (Yanighara, Sakata, Shoyama, & Murakami, 1996). We therefore collected different parts of each plant, leaf, fruit, seed, and/or bark for our first-line screening. Another report showed that the concentration of plant components was partly dependent on the growth temperature (Shiping, Shan, Tanaka, & Shoyama, 1998). Collection was therefore done in two separate locations to compare the effect of climate on the antiprotozoan activity of the selected plant species. In this study, Ghanaian medicinal plants, with antiviral, antiparasitic, and antibacteria properties, were selected based on traditional knowledge. One hundred twelve ethanolic extracts representing 72 plant species belonging to 38 families were screened for their antiprotozoan activities against *Trypanosoma*, *Leishmania*, and *Plasmodium* parasites. Although many of them have previously been reported to be effective against protozoan, diarrheal, bacterial, and other infectious diseases (Adepiti, Adewunmi, & Agbedahunsi, 2014; Bizimana et al., 2006; Chakraborty, Gaikwad, & Singh, 2012; Deepa & Rajendran, 2007; Lim, 2012; Mesia et al., 2007; Mothana et al., 2010; Mukhtar et al., 2008; Musuyu Muganza et al., 2012; Namukobe et al., 2011; Phillipson, 2001; Ravikumar, Inbaneson, Suganthi, Gokulakrishnan, & Venkatesan, 2011; Ríos & Recio, 2005; Sundararajan et al., 2006; Tajudeen & Kuranga, 2013; Yadav & Agarwala, 2011), we report the antiprotozoan activity of many crude extracts for the first time.

We confirmed high anti-*Trypanosoma* activity in 11 crude extracts, from 10 plants, with IC_{50} values less than 20 μg/ml: *A. hispidum* (whole plant), *Bidens pilosa* (whole plant), *C. cordifolia* (leaves), *Eugenia* species (seeds), *M. lucida* (leaves), *Newbouldia laevis* (leaves), *Piper guineense* (seeds), *Pycnanthus angolensis* (stem bark), *T. ivorensis* (stem bark and leaves), and *Z. zanthoxyloides* (stem bark). This study reports the anti-*Trypanosoma* activity of three of the 10 plant species, *C. cordifolia*, *N. laevis*, and *P. angolensis*, for the first time. The anti-*Trypanosoma* activity of the remaining seven plant species have been reported in previous studies (Abedo et al., 2013; Bero, Hannaert, Chataigné, Hérent, & Quenti-Leclercq, 2011; Ganfon et al., 2012; Kimani, Gathumbi, Auma, Ngeranwa, & Masiga, 2013; Mann et al., 2011; Shuaibu et al., 2008). The possible mechanisms of action are however unknown.

M. lucida was the only extract observed to induce significant apoptosis-like cell death in *Trypanosoma* parasites through the externalization of phosphotidyl serine. Subsequent bioassay-guided fractionation of *M. lucida* leaf extracts led to the identification of three novel tetracyclic iridoid compounds, two of which induced significant apoptosis-like cell death in *Trypanosoma* parasites in vitro (Karasawa et al., 2016; Kwofie et al., 2016; Suzuki et al., 2015). Thus, these compounds may be responsible for the apoptosis inducing properties of the *M. lucida* leaf extract in *Trypanosoma* parasites. Although *M. lucida* leaf extracts showed neither anti-*Leishmania* nor anti-*Plasmodium* activity, two of the three compounds had anti-*Leishmania* activity whereas all three had anti-*Plasmodium* activity.

In the anti-*Leishmania* activity screening, the promastigote form of the parasite was used. This is because *Leishmania* promastigotes are easier to handle making them more convenient for first-line screening of a large number of extracts or compounds. Moreover, extracts with

high anti-*Leishmania* promastigote activity have been reported to have higher activity against the amastigote form of the parasite (Amoa-Bosompem et al., 2016). Extensive work has however been done on selected active fractions and compounds using both the promastigote (Amoa-Bosompem et al., 2016) and amastigote forms of the parasite (unpublished). In our first-line screening however, we confirmed *A. senegalensis* (leaves), *C. alata* (leaves), *C. anisata* (roots), *B. ferruginea* (leaves), *C. cordifolia* (leaves), *Thonningia sanguinea* (whole plant), *P. clappertoniana* (stem bark and leaves), and *Z. zanthoxyloides* (root) as extracts with strong anti-*Leishmania* activity. This study reports the anti-*Leishmania* activity of six of the eight active plant species for the first time with *A. senegalensis* and *Z. zanthoxyloides*, the only ones previously reported to possess anti-*Leishmania* activity (Sahpaz et al., 1994). With respect to extract activity against multiple parasites, *C. cordifolia* and *Z. zanthoxyloides* were found to have activity against both *Trypanosoma* and *Leishmania* parasites. The *Z. zanthoxyloides* stem bark extract however had only anti-*Trypanosoma* activity whereas its root extract had only anti-*Leishmania* activity. This difference may be attributed to the differences in chemical components and/or concentration in different plant parts. Both *T. ivorensis* stem bark and leaf extracts were active against *Trypanosoma* and *Plasmodium* parasites.

In addition to the anti-*Leishmania* activity, we found *A. senegalensis* to induce kinetoplast disintegration in deformed *Leishmania* parasites although there was no observable effect on the nucleus. The shift from the normal morphology may be due to the minor aggregation of parasites resulting in the inhibition of parasite proliferation. Nuclear fragmentation was observed in *Leishmania* parasites treated with *B. ferruginea*, *P. clappertoniana*, or *C. cordifolia* extracts. *C. alata*-treated parasites formed the largest aggregation relative to all the test groups with a significant increase in the number of parasites having a double nuclei and kinetoplast. This phenotype may be due to the inhibition of cytokinesis after nuclei and kinetoplast division, preventing the formation of two distinct daughter cells. *Z. zanthoxyloides* extract on the other hand caused minor aggregation in *Leishmania* parasites with a short and stumpy-like morphology. All the anti-*Leishmania* active extracts did not induce significant apoptosis in *Leishmania* parasites (data not shown).

With respect to the anti-*Plasmodium* activity, six extracts from five plant species were observed to have high anti-*Plasmodium* activity: *Alchornea cordifolia* (leaves), *Anogeissus schimperi* (leaves), *Gossypium arboretum* (leaves), *Mangifera indica* (leaves), and *T. ivorensis* (stem bark and leaves). Although all five plant species had previously been reported to have anti-*Plasmodium* activity, the plant parts, extracts, differed in activity from previous reports. The leaves, flowers, and bark of *M. indica*, for example, had previously been reported to have anti-*Plasmodium* activity (Bidla et al., 2004; H. A. Ibrahim et al., 2012). Our study however found only the leaf extracts to have anti-*Plasmodium* activity (Table 2). This may be due to the effect of seasons and/or habitats on the constituents of plants. Regarding the components of mango, although it has been suggested that the most important constituent in mango-related antiparasitic activity is mangiferin having the C-glucosyl xanthone structure (Wauthoz & Balde, 2007), the active component is still unknown. In this regard, we intend to employ the bioassay-guided fractionation techniques to determine the active components of the promising anti-*Plasmodium* active extracts as in the case of *M. lucida* (Kwofie et al., 2016).

Moving forward, our aim is not only to determine the active components but also to test the prospects of using active extracts to develop herbal based treatment drugs at the Center for Plant Medicine Research, Ghana. Work is currently ongoing to replicate the efficacy of selected active extracts and compounds in vivo. Also, although climate had no significant effect on the activity of active extracts, there is the need to determine the seasonal activity of each/selected active extracts. This study however shows the prospects of Ghanaian medicinal plants in the development of new chemotherapy against protozoan infections.

5 | CONCLUSION

In conclusion, all 23 extracts with high antiparasitic activity showed high selectivity for at least one of the three parasites. Anti-*Trypanosoma* active compounds induced apoptosis in *Trypanosoma* parasites whereas anti-*Leishmania* active extracts caused morphological changes in the *Leishmania* parasite.

Overall, the results obtained from the crude extracts screening, especially all extracts with anti-*Trypanosoma*, anti-*Leishmania*, and anti-*Plasmodium* activities, suggest that these may be promising sources for the development of new drugs for controlling African trypanosomiasis, leishmaniasis, and malaria.

ETHICS APPROVAL AND CONSENT TO PARTICIPANTS

IRB approval was sought from the Noguchi Memorial Institute for Medical Research, Ghana, IRB board before the start of the project.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIAL

All data generated in this study have been included in this manuscript.

AUTHOR CONTRIBUTIONS

O. M. developed protocols, performed assays, analyzed data, and was a major contributor in the writing of the manuscript. K. D. K., A. B. M., and A. J. performed antiparasitic assays, analyzed data, and contributed to the writing of the manuscript. U. T., A. R., S. M., A. F., A. F., and A. A. A. prepared the plant extracts. O. K. B. A., T. I., A. P., N. T., N. A., and A. O. R. performed toxicity assays and analyzed data. Y. S. is responsible for the authentication of plant material and contributed to the writing of the manuscript. W. K. A., A. I., B. D. A., K. K. A., E. D., S. Y., and O. N. analyzed data and contributed to the writing of the manuscript. All authors have read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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