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# Antimicrobial and antiprotozoal activities of twenty-four Nigerian medicinal plant extracts

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ABSTRACT

Medicinal plants exploration has become an important tool in the discovery of bioactive natural substances capable of inhibiting the mechanisms of microbial and protozoal activity. The aim of this study was to determine the antimicrobial and antiprotozoal activities of medicinal plants extracts used for the treatment of various illnesses in southwestern Nigeria.

Twenty-four medicinal plant extracts were screened for antimicrobial activity against three fungal and six bacterial strains; and antiprotozoal activities against chloroquine-sensitive strains of *Plasmodium falciparum* (antiplasmodial assay), a culture of *Leishmania donovani* promastigotes and axenic amastigotes (antileishmanial activity) and two-days old culture of *Trypanosoma brucei brucei* (antitrypanosomal assay).

Five extracts exhibited strong antifungal activity against *Cryptococcus neoformans*, with *Ricinodendron heudelotii* (Baill.) Heckel, *Terminalia ivorensis* A.Chev and *Macaranga barteri* Müll. Arg, having an $IC_{50}$ of 31.73, 32.10 and 75.63 μg/mL, respectively. Ten of the extracts were active against *T. brucei*, with *Eleusine indica* displaying the most significant activity ($IC_{50}$ and $IC_{90}$ of 8.26 and 10.14 μg/mL). None of the extracts displayed any significant antiplasmodial and antileishmanial activities. In addition, none of the extracts displayed cytotoxicity on transformed human monocytic (THP1) cells.

The study revealed that *M. barteri* had the broadest spectrum of activity, with activity against *C. neoformans*, *P. aeruginosa*, vancomycin resistant *Enterococci faecalis* (VRE) and *T. brucei*. *M. barteri* could be exploited for broad spectrum antimicrobial and antitrypanosomal activity, while *Eleusine indica* could be subjected to bioassay guided fractionation and isolation of antitrypanosomal constituents. This study suggests that the evaluated plants are potential sources of novel anti-infective agents.



## 1. Introduction

Infectious diseases are one of the most important factors responsible for the high rate of morbidity and mortality, especially in developing countries, where over 25% of global annual mortality are primarily caused by infectious diseases (Mahady, 2005). Meanwhile, secondary effects of infections are responsible for millions more deaths (Satcher, 1995; Chua and Gubler, 2013). Human African trypanosomiasis (HAT), Chagas diseases, leishmaniasis and malaria are infectious diseases caused by protozoans. HAT, Chagas diseases and leishmaniasis are classified as neglected tropical diseases (NTDs) by the World Health Organization (WHO) and they represent a major threat to the health and well-being of over a billion lives worldwide (WHO, 2010; Llurba-Montesino et al., 2015).

Over the years, significant progress has been made in the field of microbiology and the control of microorganisms. In spite of this progress, sporadic episodes of epidemics triggered by drug-resistant microorganisms and pathogenic microbes remain a massive threat to public health. The relentless rise in antibiotic resistance, and emergence of new and chronic infectious diseases are factors that stimulate a continuous search for new anti-infective agents (Costa et al., 2016; Mahady, 2005; Al-Judaibi, 2014; Mehani et al., 2016).

Plant-based traditional medicine represents the primary or the only veritable form of accessible primary health care in many parts of the developing world, especially for people living in rural Africa. The information about the healing properties of medicinal plants have been transmitted over the centuries among generations of human communities; the existence of traditional medicine depends on plant species diversity and the knowledge of their use as herbal medicine. Currently, the demand for the herbal drug treatment of various ailments is increasing and plants are being explored globally for the development of newer drugs (Silva and Fernandes, 2010; Mahomoodally, 2013).

Considering the importance of medicinal plants for the discovery and identification of bioactive natural substances capable of inhibiting the

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mechanisms of microbial activity and resistance (Amoa-Bosompem, 2016; Nguyen, 2016), this work reports the evaluation of twenty-four extracts from medicinal plants utilized in ethnobotanical medicine in Nigeria (Ajaiyeoba et al., 2003; Osowole et al., 2005; Ogbole et al., 2010; Segun et al., 2018) against agents responsible for various microbial infections including malaria, leishmaniasis and trypanosomiasis.

## 2. Materials and methods

### 2.1. Plant collection

Fresh leaves of the medicinal plants were collected from their various habitat at the onset of raining season in Ibadan, Nigeria between April and May 2015. The plants were identified and authenticated by Dr. Osiyemi of the Forestry and Research Institute of Nigeria (FRIN), Ibadan and assigned voucher specimen numbers. Voucher specimens were deposited at the Forest Herbarium, Ibadan (FHI).

### 2.2. Preparation and extraction of plant materials

Each plant material was shade-dried at room temperature (27–33 °C) and pulverized. The dried powdered materials of each plant (100 g) were extracted by maceration into methanol (750 mL) and then filtered to obtain methanol extracts of each plant. The filtrate was concentrated with a rotary evaporator at 40 °C and the dried extracts were kept in a refrigerator until needed. Even though water is mostly used as solvent base for traditional herbal preparation, methanol was used in this study, mainly because of its amphiphilicity; it dissolves a wider range of compounds than water, including polar and to a large extent some non-polar compounds. Moreover, methanol easily evaporates so it is easier to separate from the extract than water.

### 2.3. Antimicrobial assay

Antimicrobial susceptibility testing was carried out at the National Center for Natural Products Research, University of Mississippi, USA. The test organisms were obtained from the American Type Culture Collection (ATCC, Manassas, VA). These included the fungi: *Candida albicans* ATCC 90028, *Cryptococcus neoformans* ATCC 90113 and *Aspergillus fumigatus* ATCC 204305; bacteria: *Staphylococcus aureus* ATCC 29213, methicillin-resistant *Staphylococcus aureus* ATCC 33591 (MRSA), *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 4352 and Vancomycin resistant *Enterococci faecalis* ATCC 29212 (VRE) The *in vitro* antimicrobial assay was done using a modified version of the CLSI methods as described below (Samoylenko et al., 2009). Extracts were dissolved in dimethyl sulfoxide (DMSO) and serially diluted in 20% DMSO/saline and then transferred, in duplicate, into 96-well flat bottom microplates. The final concentration of DMSO used in this assay was 0.1%, as concentration below 1% is not known to exert any observable toxic effect on cells (Timm et al., 2013). Microbial innocula were prepared in assay medium to afford target $10^6$ cfu/mL, after addition to the samples. Growth, solvent and media controls were included in each test plate. Assay plates were read at 530 nm before and after incubation using the Biotek Power wave XS plate reader. Percentage growth was plotted against test concentration to afford the $IC_{50}$ values (concentration that affords 50% growth relative to control). Crude plant extracts were tested in the primary assay at a single concentration of 200 μg/mL and percentage inhibitions were calculated, extracts with more than 50% inhibition were subjected to the secondary screening using serially diluted extract at concentration range of 200–8 μg/mL. Drug controls were included in the secondary assay; ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and amphotericin B (ICN Biomedicals, Ohio) for fungi.

### 2.4. Antiplasmodial assay

Antiplasmodial activities were measured *in vitro* using the assay protocol based on a colorimetric method that determines the parasitic lactate dehydrogenase (pLDH) activity (Makler et al., 1993; Samoylenko et al., 2009). The assay was performed in 96-well microplate and included a chloroquine-sensitive strains of *Plasmodium falciparum* (D6). In the primary screening, the crude plant extracts were tested in duplicate, at a single concentration of 15.9 μg/mL on the chloroquine-sensitive (D6) strain of *P. falciparum*. DMSO and chloroquine were included as vehicle and drug control, respectively.

### 2.5. In vitro assay for antileishmanial activity

Alamar blue assay was used to evaluate the antileishmanial activity of the plant extracts. The assay was carried out on a culture of *Leishmania donovani* promastigotes and axenic amastigotes (Mikus and Steverding, 2000). The promastigotes culture was maintained at 26 °C in Roswell Park Memorial Institute medium (RPMI 1640), pH 7.4 with 10% fetal bovine serum (FBS). The axenic amastigotes were cultured at 37 °C and 5% $CO_2$ in RPMI-1640 supplemented with 4-morpholineethanesulfonic acid (MES) (4.88 g/L), L-glutamine (298.2 mg/L), adenosine (26.7 mg/L), folic acid (10.1 mg/L), BME vitamin mix, sodium bicarbonate (352.8 mg/L) and 10% FBS. The pH of the culture medium was 5.5. For the primary assay, plant extracts were tested at a single concentration of 20.0 μg/mL (in duplicate) on both the promastigotes and axenic amastigotes culture. The extracts that showed more than 90% inhibition of growth in primary screening were subjected to secondary screening for dose response analysis. In a 96-well microplate, appropriate dilutions of samples, were added to the *Leishmania* promastigotes/axenic amastigote culture ($2 \times 10^6$ cell $mL^{-1}$). The extracts were tested at six concentrations ranging from 40 to 0.0128 μg/mL. The plates were incubated for 72 h at 26 °C and 37 °C, respectively, for promastigotes and axenic amastigotes. The growth of *Leishmania* promastigotes/amastigotes was determined. $IC_{50}$ and $IC_{90}$ values were computed from the dose–response curves (Donega et al., 2014).

### 2.6. Antitrypanosomal assay

Two-day old culture of *Trypanosoma brucei*, in the exponential growth stage, was diluted with Iscove's Modified Dulbecco's medium (IMDM) to obtain 5000 parasites/mL. The assays were conducted in 96-well microplates. Extract dilutions (1 mg/mL) were made in IMDM from the stock extracts (20 mg/mL) for the primary screening. Each well received 4 μL of diluted extract and 196 μL of the culture medium to obtain a final volume of 200 μL. Plates were incubated at 37 °C in 5% $CO_2$ for 48 h. Ten microliter (10 μL) of alamar blue (AbD Serotec, catalog number BUF012B) was added into each well and plates were then incubated overnight. Standard fluorescence was measured on a Fluostar Galaxy fluorometer (BMG LabTechnologies) at 544 nm excitation and 590 nm emission. Pentamidine was included as the control drug (Table 4). Extracts with more than 90% inhibition of *T. brucei* growth in primary screening were subjected to secondary screening to evaluate dose–response analysis. They were screened at concentrations ranging from 10 to 0.4 μg/mL. $IC_{50}$ and $IC_{90}$ values were computed from dose response growth inhibition curve by XLfit version 5.2.2. (Jain et al., 2016).

### 2.7. Cytotoxicity assay

The extracts were screened for cytotoxicity against transformed human monocytic (THP1) cells. Four-day old culture of THP1 cells was diluted with RPMI medium to $2.5 \times 10^5$ cells/mL. For transformation of the cells to adherent macrophages, phorbol 12-myristate 13-acetate (PMA) was added to the culture at 25 ng/mL concentration