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Bioassay-guided isolation of active principles from Nigerian medicinal plants identifies new trypanocides with low toxicity and no cross-resistance to diamidines and arsenicals

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ABSTRACT

Ethnopharmacological relevance: Leaves from the plant species studied herein are traditionally used in northern Nigeria against various protozoan infections. However, none of these herbal preparations have been standardized, nor have their toxicity to mammalian cells been investigated. In search of improved and non-toxic active antiprotozoal principles that are not cross-resistant with current anti-parasitics, we here report the results of the *in vitro* screening of extracts from seven selected medicinal plant species (*Centrosema pubescens*, *Moringa oleifera*, *Tridax procumbens*, *Polyalthia longifolia*, *Newbouldia laevis*, *Eucalyptus maculate*, *Jathropha tanjorensis*), used traditionally to treat kinetoplastid infections in Nigeria, and the isolation of their bioactive principles.

Aim of the study: To investigate the efficacies of medicinal plant extracts, and of compounds isolated therefrom, against kinetoplastid parasites, assess cross-resistance to existing chemotherapy, and assay their toxicity against mammalian cells *in vitro*.

Material and methods: Plants were extracted with hexane, ethyl acetate and methanol. Active principles were isolated by bioassay-led fractionation, testing for trypanocidal activity, and identified using NMR and mass spectrometry. EC_{50} values for their activity against wild-type and multi-drug resistant *Trypanosoma brucei* were obtained using the viability indicator dye resazurin.

Results: Seven medicinal plants were evaluated for activity against selected kinetoplastid parasites. The result shows that crude extracts and isolated active compounds from *Polyalthia longifolia* and *Eucalyptus maculata*, in particular, display promising activity against drug-sensitive and multi-drug resistant *Trypanosoma brucei*. The EC_{50} value of a clerodane (16α-hydroxy-cleroda-3,13(14)-Z-dien-15,16-olide) isolated from *Polyalthia longifolia* was as low as 0.38 μg/mL, while a triterpenoid (3β,13β-dihydroxy-urs-11-en-28-oic acid) isolated from *Eucalyptus maculata* displayed an EC_{50} of 1.58 μg/mL. None of the isolated compounds displayed toxicity towards Human Embryonic Kidney cells at concentrations up to 400 μg/mL. In addition, the isolated compounds were active against *Leishmania mexicana*, as well as against *T. congolense*.

Conclusion: We have isolated a clerodane compound from *Polyalthia longifolia* that shows low toxicity, no cross-resistance with current treatments, and promising activity against both human-infective and veterinary *Trypanosoma* species.

1. Introduction

Infectious diseases that are caused by certain species of the genera Trypanosoma (Human African trypanosomiasis or HAT, and Chagas disease) and Leishmania (various forms of leishmaniasis) are amongst the neglected tropical disease, affecting many millions of people

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throughout the tropics. Mortality and morbidity resulting from these diseases is still very high in developing countries (Hotez and Kamath, 2009; Hotez et al., 2009). New and improved treatments would undoubtedly enhance the welfare of the local population and livestock (Nwodo et al., 2015; Giordani et al., 2016). While mortality due to HAT is clearly important, African Animal Trypanosomiasis (AAT or nagana) is considered the livestock disease with the highest impact on agricultural production and animal husbandry in Africa, causing annual losses which run to billions of US dollars (Samdi et al., 2010). Across the tsetse belt as many as 55 million heads of cattle are at risk of infection, plus 30 million sheep and 40 million goats. It is estimated that 3 million cattle die every year from African trypanosomiasis (Samdi et al., 2010; Seyoum et al., 2013).

African trypanosomiasis and leishmaniasis as global health challenges are compounded by the lack of vaccines, making chemotherapy the only suitable alternative at the moment (La Greca and Magez, 2011). In addition, existing antiparasitic drugs are hampered by toxic side effects and the emergence of resistance (Delespaux and De Koning, 2007; Fairlamb et al., 2016). However, natural products have been identified as highly promising starting points for the discovery of anti-protozoan agents (e.g. Salem and Werbovetz, 2006; Omar et al., 2016; Siheri et al., 2016; Dike et al., 2016), and indeed have a long and distinguished history as essential drugs in the fight against tropical disease (e. g. Tu, 2011). Indeed, over the period 1981–2002, 61% of all new chemical entities approved for infectious diseases were natural compounds or directly derived thereof (Newman et al., 2003).

Natural products derived from plants have played an important role in the control of diseases caused by the protozoal parasites, a classical example is malaria caused by *Plasmodium falciparum*. Current malaria treatment relies heavily on plant-derived products, including the sesquiterpene lactone artemisinin (Wells, 2011), and the alkaloid quinine (Moyo et al., 2016). The success stories of artemisinin, isolated from the Chinese wormwood plant (*Artemisia annua*) and of quinine, isolated from the bark of cinchona trees (*Cinchona officinalis*), both used traditionally as antimalarial therapies, justify drug discovery based on ethnopharmacological usage, as these drugs were discovered following an intensive screening of hundreds of plants traditionally used for treating malaria (Tu, 2011). These achievements notwithstanding, unfortunately, fewer efforts have been put into investigating extracts from various plants traditionally used for treating the kinetoplastid diseases like trypanosomiasis and leishmaniasis despite the undesirable side effects and marginal effectiveness of the currently available drugs.

Centrosema pubescens, Moringa oleifera, Tridax procumbens, Polyalthia longifolia, Newbouldia laevis, and *Eucalyptus maculata* thrive well in many areas of Nigeria where they grow in the wild except for *Moringa oleifera,* which is cultivated for food and for medicinal use. *Newbouldia laevis* is mostly used in northern Nigeria for boundary demarcation, while *Polyalthia longifolia* commonly called "mast tree" is used as an ornamental plant. These plants are highly valued and are also used for treating various infections caused by the protozoan parasites including species of *Trypanosoma, Leishmania, and Plasmodium*, especially the strains that resists existing chemotypes (Tor-Anyiin et al., 2003; Igoli et al., 2004, 2005; Alli et al., 2011; Abubakar et al., 2012; Nwodo et al., 2015; Bankole et al., 2016). Unfortunately none of the herbal preparations from these plants have been standardized for use, nor extensively investigated for their toxicity on mammalian cells, and their activity against protozoan parasites has not been scientifically validated. Because these plants grow on many soil types, are abundant in the wild, and only their leaves are used, their utilization as herbal remedies pose no danger to biodiversity.

The development of an effective chemotherapy based on a local resource will ensure new drugs that will both address the urgent need for new treatments and provide an additional economic driver for poor farming communities. In this study, we investigate the anti-kinetoplastid potential of selected herbs traditionally used for treating infections caused by trypanosomatid parasites in northern Nigeria.

2. Materials and methods

2.1. Plant selection and collection

In Nigeria, the use of traditional herbal medicine is part of the unique tradition acceptable to the majority of the people (Sofowora, 1982; Heinrich, 2000; Adetutu et al., 2011). This notwithstanding, the secrecy associated with this practice has led to a paucity of scientific reporting of this knowledge in literature (Ashidi et al., 2010). However, the selection of traditional medicinal plants used in the present study is based on interviews with traditional healers who were willing to divulge the information. Following the method of Adetutu et al. (2011), ethnopharmacological methods were used to select the plants used in this research.

Briefly, the selection followed interviews with the traditional healers, using structured questionnaires to establish which plant and part of the plant is in common use against tropical fevers. Thirty-one traditional healers were approached, with eighteen volunteering to complete the survey. The information collected included local names of the plants used for treating various forms of fevers, parts of the plant commonly used, methods of preparation, and details of administration. The frequency of citation of each plant was recorded. Seven plants were consistently cited. The selected plants were purchased from the healers and their specimens were used for identification at the herbarium of Kogi State University, Nigeria. Each plant name has been checked with www.theplantlist.org and this website was last accessed on the 06/01/2017. The plants were identified as shown in Table 1:

Plant collection was carried out in the months of January and February 2014 from the town of Anyigba, Kogi State, Nigeria (latitude 7° 15′–7° 29′ N and longitude 7° 11′–7° 32′ E; altitude of 410–430 m).

2.2. Extraction procedure

The leaves of the plants were thoroughly air dried and subsequently were ground to a fine power using a grinder before extraction.

The powdered dry leaves (20 g) were weighed into an extraction thimble (Fisher Scientific) and placed in a Soxhlet apparatus. The plant materials were extracted consecutively using solvents of increasing polarity starting with *n*-hexane and followed by ethyl acetate and methanol (500 mL of each solvent; all obtained from Fisher Scientific (Loughborough, UK)). Each extraction stage was carried out to exhaustion. All extracts obtained were evaporated at 40 °C; recovery of solvent, and concentration of the extracts, was carried out under vacuum using a rotary evaporator connected to a condenser. Residual solvents were further allowed to evaporate under the fume hood before samples were freeze dried in a freeze dryer. The hexane, ethyl acetate, and methanol extracts obtained were labelled as below, then stored at −20 °C prior to analysis. The yields of the crude extracts were between 2 and 5 g.

Table 1
Botanical name and voucher number for each plant evaluated in this study.

Code	Botanical name	Voucher number
A	*Centrosema pubescens* Benth. (Family *Fabaceae*)	0446
B	*Moringa oleifera* Lam (Family *Moringaceae*)	0447
C	*Tridax procumbens* (L.) L. (Family *Compositae*)	0448
D	*Polyalthia longifolia* (Sonn.) Thwaites (Family *Annonaceae*)	0449
E	*Newbouldia laevis* (P.Beauv.) Seem. (Family *Bignoniaceae*)	0450
F	*Eucalyptus maculata* Hook. (Family *Myrtaceae*)	0451
G	*Jatropha tanjorensis* J.L.Ellis & Saroja (Family *Euphorbiaceae*)	0452