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Antiplasmodial activity and phytochemical analysis of extracts from selected Ugandan medicinal plants

Madina Mohamed Adia [a,*], Seyedeh Noushin Emami [b], Robert Byamukama [a], Ingrid Faye [b], Anna-Karin Borg-Karlson [c]

[a] Department of Chemistry, Makerere University, P.O. Box 7062, Kampala, Uganda
[b] Department of Molecular Biosciences, The Wenner-Gren Institute (MBW) Stockholm University, Stockholm, Sweden
[c] Department of Chemistry, School of Chemical Science and Engineering, Kungliga Tekniska Hogskolan (KTH), Stockholm, Sweden

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ABSTRACT

Ethnopharmacological relevance: Resistance of the parasites to known antimalarial drugs has provided the necessity to find new drugs from natural products against malaria. The aim of the study was to evaluate the *in vitro* antiplasmodial activity of some plants used by Traditional Medical Practitioners (TMPs) of Prometra and Rukararwe in malaria treatment in Uganda to provide scientific proof of the efficacies claimed by these Herbalists.

Materials and methods: The air dried samples of *Clerodendrum rotundifolium* (leaves), *Microglossa pyrifolia* (leaves), *Momordica foetida* (leaves) and *Zanthoxylum chalybeum* (stem bark) used for malaria treatment by TMPs were successively extracted with ethyl acetate, methanol and water to yield twelve extracts. The extracts were tested against the chloroquine-sensitive (NF54) and chloroquine-resistant (FCR3) *Plasmodium falciparum* strains *in vitro* using the micro Mark III test which is based on assessing the inhibition of schizont maturation. A compound A was extracted and purified from the stem bark of *Z. chalybeum* and its structure was identified and confirmed by spectroscopic methods.

Results: Most of the extracts tested (92%) showed an antiplasmodial activity with $IC_{50} < 50$ μg/mL. In spite of successive extractions with different solvents, potent anti-plasmodial activity ($IC_{50} < 5$ μg/mL) was observed in the ethyl acetate, methanol and aqueous extracts of *M. pyrifolia* and *C. rotundifolium*. Preferential enrichments of activity into water ($IC_{50} < 15$ μg/mL) and Ethyl acetate ($IC_{50} < 5$ μg/mL) were seen in the case of *M. foetida* and *Z. chalybeum* respectively. The most active extracts were from *C. rotundifolium* and *M. pyrifolia* with IC_{50} values less than 2 μg/mL. Phytochemical analysis of the extracts revealed the presence of saponins, tannins, flavonoids, alkaloids and cardiac glycocides. Fagaramide isolated from *Z. chalybeum* had a higher activity (IC_{50} 2.85 μg/mL) against the chloroquine-resistant strain than against the chloroquine-senstive (IC_{50} 16.6 μg/mL) strain used in the study.

Conclusion: The plant extracts analysed in this study presented an average antiplasmodial activity (58%). This study revealed for the first time the antiplasmodial activity of the plant *C. rotundofolium*. It's the first time the compound fagaramide (N-isobutyl-3-(3,4-methylene dioxyphenyl)−2E-propenamide) has been isolated from *Z. chalybeum* as one of the compounds that contribute to the activity of this plant against *P. falciparum*.

1. Introduction

Malaria is an infectious disease and can be lethal if not diagnosed and treated early. Half of the world's population was estimated to be at risk of malaria by 2012 (WHO, 2014). The African region bears the highest burden of malaria, with 80% of the estimated 207 million cases and 90% of the estimated 627,000 malaria deaths worldwide occurring in Africa (WHO, 2014). Seventy seven percent of all malaria deaths occur in children under five years of age (WHO, 2014).

The World's poorest people are the most affected with malaria and many of them get treatment from traditional medicines because they are readily available and cheap compared to conventional medicine. Some local communities perceive traditional medicine as more effective than conventional medicine and Traditional Medical Practitioners (TMPs) use herbal remedies for treatment of malaria in Uganda (Adia et al., 2014). Missing gaps in scientific evidence of the efficacies of some plants claimed by the

* Corresponding author.
E-mail addresses: madrine@cns.mak.ac.ug, madrine1@gmail.com (M.M. Adia).

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Table 1
The selected plant species and their traditional uses in Uganda.

Species/voucher specimen no./ Family	Site of collection	Part used	Local name	Traditional use
Microglossa pyrifolia (Lam.) O. Ktze (MAM 1), Asteraceae	Buyija	Leaves	Kafugankande	Malaria, abdominal disorders, cough and chest pain (Adia et al., 2014) Convulsions, skin allergy and syphilis (Asiimwe et al., 2014). Malaria (Katuura et al., 2007). Roots for malaria (Stangeland et al., 2011).
Clerodendrum rotundifolium Oliv. (MAM 2), Lamiaceae	Buyija	Leaves	Kisekeseke	Malaria and diabetes (Adia et al., 2014). Deworming and stomach ache (Asiimwe et al., 2014). Malaria (Katuura et al., 2007). Treatment of Intestinal parasites (Hamill et al., 2003). Induction of labour in childbirth (Kamatenesi-Mugisha and Oryem-Origa, 2007).
Momordica foetida Schumach (MAM 91), Cucurbitaceae	Rukararwe	Leaves	Omwihura	Baths, cough, vomiting (Adia et al., 2014). Malaria (Tabuti, 2008). Flue and worms, (Namukobe et al., 2011). Stomach ache (Hamill et al., 2003). Antimalarial activity detected (Froelich et al., 2007; Waako et al., 2005).
Zanthoxylum chalybeum Engl. (MAM 62), Rutaceae	Rukararwe	Stem bark	Mutatembwa/ Munyenye	Roots for malaria (Tabuti, 2008). Antiplasmodial activity detected in the leaves (Bbosa et al., 2014).

TMPs necessitated for this study. This study was carried out to evaluate the *in vitro* antiplasmodial activity of four Ugandan medicinal plants which were collected and selected based on their frequent use. The dominant *Plasmodium* parasite species is resistant to some standard antimalarial drugs like chloroquine. Resistance of the parasites to known antimalarial drugs has provided the necessity to find new drugs both by the synthetic industry and from natural products.

The plant species were selected according to the many times they were mentioned for use by the TMPs and scientific work that had been done on them before. The plants were identified from Makerere University Herbarium and Voucher specimen numbers were given. In Table 1, are plant parts used and the traditional uses in Uganda of the four plants under investigation. In this study, we report the antiplamodial activity of the crude extracts and of the isolated compound Fagaramide from *Z. chalybeum*.

2. Materials and methods

2.1. Plant materials

The plant species were identified by Mr. Rwaburindori Protase (the taxonomist) of Makerere University Herbarium. Voucher specimen numbers were given and Voucher specimen deposited to Makerere University Herbarium. Materials from the following plants were used; Leaves of *Clerodendrum rotundifolium* Oliv. (Voucher number: MAM 2) and leaves of *Microglossa pyrifolia (*Lam.) O. Ktze (Voucher number: MAM 1), were collected from Buyija-Buwama Mpigi district where Prometra (Promocion de la medicina tradicional amazonica) group of Traditional Healers association are found. The stem bark of *Zanthoxylum chalybeum* Engl, (Voucher number: MAM 62) and leaves of *Momordica foetida* Schumach (Voucher number: MAM 91), were collected from Rukararwe-Bumetha group of TMPs found in Bushenyi district.

2.2. Extraction of the plant materials

The air dried and powdered materials, (700 g) of each plant species were extracted with ethyl acetate (12 L) by maceration for 48 h. The residue was air dried overnight and then extracted with methanol (7 L) by maceration for 48 h. The residue was again air dried overnight and then extracted with water (4 L) by maceration for 24 h.

The extracts were filtered and evaporated to dryness using a rotary evaporator (B"U" CHI-SWITZERLAND) with freeze drier for the ethyl acetate, methanol and water extracts respectively to give the crude extracts. The dried extracts were stored in the freezer at -12 °C until used.

2.3. Phytochemical analysis of the plant extracts

Phytochemical screening of the plant extracts was carried out using standard conventional protocols of Evans (2002), Sofowara (1993) and Harborne, (1973) for detecting the major components. Appearance of froth after shaking and allowing to stand for 10 min on addition of distilled water to the powder indicated presence of saponins. The presence of cardiac glycocides was detected by addition of Fehling's solution followed by concentrated sulphuric acid to the powder and solution turns greenish blue. Also about 100 mg of extract dissolved in 1 mL of glacial acetic acid containing one drop of Fehling's solution, then one mL of concentrated sulphuric acid added without shaking to form two layers, appearance of a brown ring at the interface indicates the presence of a de-oxy sugar characteristic of cardenolides (Keller Killiani's test). Blue-black or blue-green colouration observed on addition of 3 mL of 10% Ferric chloride solution to 1 mL of the plant extract was used for indication of the presence of gallic and catechol tannins respectively. The presence of flavonoids was detected by yellow solution turning colourless on addition of dilute HCl to 0.2 g of powdered sample in dilute NaOH solution. Reducing sugars were detected by solution turning dark green on addition of 6 drops of Fehling's solution to 1 mL of the extract solution and alkaloids were detected by appearance of white-yellow precipitate on addition of Mayer's reagent.

2.4. Isolation of compounds from Zanthoxylum chalybeum Engl

The Ethyl acetate crude extract of *Z. chalybeum* was subjected to medium pressure liquid chromatography packed with silica gel (35–70 μM) using a gradient system of hexane-ethyl acetate and ethyl acetate–methanol to yield 20 fractions (1–20). Analytical TLC was carried out on aluminium plates precoated with silica gel $60F_{254}$ 15 μM, 0.2 mm (Merck). The spots were visualized under UV light (254 nm) and heating after spraying with vanillin reagent. PTLC was done using TLC glass plates prepared with silica gel 60 F_{254}, 0.5 mm (EDM/Merck KGaA). Fraction 15 was subjected to further purification by PTLC using DCM/MeOH 90/10 v/v solvent system to yield 30.2 mg of compound A.

2.5. Spectrometric analysis

The NMR spectra of the isolated compound were recorded on a Bruker 500 MHz NMR instrument and the sample was dissolved in chloroform-d_1. MS data was obtained on an LC-MS spectrometer auto sampler plus (FINNIGAN SURVEYOR).

2.6. Structure determination

Fagaramide (A): white needle-like crystalline solid: ^{1}H NMR (500 MHz, CDcl$_3$): δ_H 2.51(H-1), 3.40 (H-2), 4.78(H-3), 7.55 (H-13), 7.80 (H-6), 8.35 (H-8), 8.57 (H-11), 8.83 (H-12), 9.10(H-5), 2.51(H-14). ^{13}C NMR (125 MHz, CDcl$_3$): δ_C 20.35(C-1), 28.80 (C-2), 47.29 (C-3), 101.59 (C-13), 106.6 (C-8), 108.90 (C-6), C118.93(C-11), 123.94 (C-12), 129.53 (C-7), 140.85 (C-5), 148.45(C-9), 149.16(C-10), 166.2 (C-4). ESI-MS (positive ion mode) m/z $[M+H]^+$ (calcd for $C_{14}H_{17}O_3N$).

2.7. Antiplasmodial tests

P. falciparum NF54 chloroquine-sensitive strain from a patient in Netherlands, (van Schalkwyk et al., 2013) and FCR3 chloroquine-resistant strain of Gambian origin collected and kept in deep freezer under liquid nitrogen at Stockholm University, Department of Molecular Biosciences were used in the antiplasmodial assay.