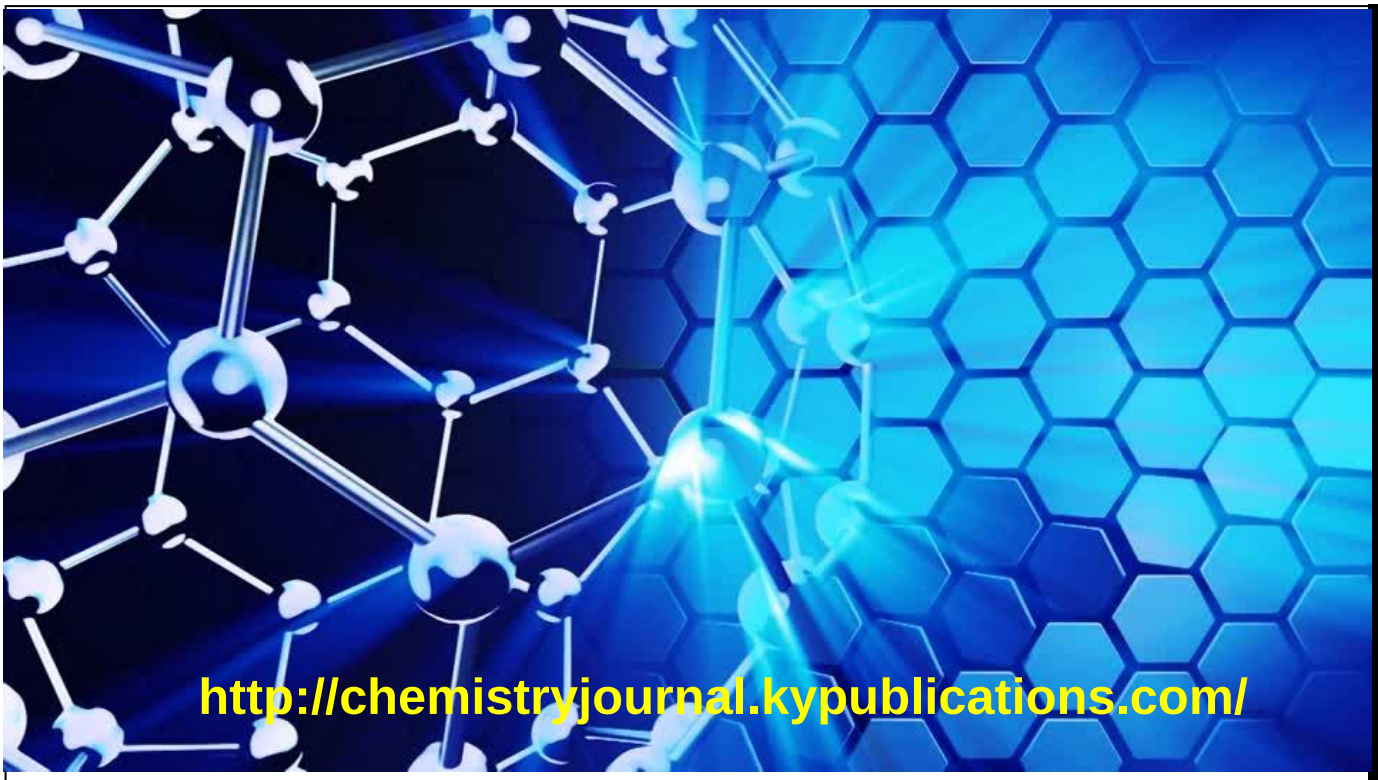

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ANTI-PLASMODIAL INVESTIGATIONS OF SELECTED CONGOLESE MEDICINAL PLANTS

RESEARCH ARTICLE

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ABSTRACT

This study reports the in vitro evaluation of extracts from eight (8) African medicinal plants used in Congolese traditional medicine for the treatment of malaria. Fourty-two (42) organic and aqueous extracts from *Alchornea cordifolia*, *Caloncoba welwitschii*, *Cnestis ferruginea*, *Dacryodes edulis, Hymenocardia acida*, *Manotes pruinosa*, *Nauclea latifolia*, and *Percea americana*, were obtained by maceration and percolation of dried and powdered plants materials. The antiplasmodial activity of the extracts was evaluated against the chloroquine sensitive D10 strain of *P. falciparum*. In general, most of the samples were inactive at the concentration tested. Among the 42 tested extracts, those originating from *Cnestis ferruginea*, *Nauclea latifolia*, *Dacryodes edulis*, *Alchornea cordifolia*, and *Caloncoba welwitschii* showed moderate activity with IC_{50} values ranging from 17.5 to 24.0 µg/mL. *D. edulis* stem bark organic extract and *A. cordifolia* leaf aqueous extract were the most active extracts, with IC_{50} values of 17.5 µg/mL.. The obtained results may partly justify and support the traditional use of the investigated plants in the treatment of malaria. Further studies for the isolation, identification and/or characterisation of individual chemical compounds from the plants under study are envisaged and could lead to significant results.

Keywords: Malaria, *P. falciparum*, anti-plasmodial, medicinal plants.

1. INTRODUCTION

Higher plants are both a fundamental key source of new chemical diversity and an integrated component of today's pharmaceutical arsenal. Throughout human history, natural products have been the foundation for the discovery and development of therapeutics used to treat ailments ranging from infectious and cardiovascular diseases to cancer. Their chemical diversity and complexity have provided structural scaffolds for small-molecule drugs and have consistently served as either the source of chemical entities introduced as drugs or the inspiration for medicinal design (Tamura et al., 2010; Nogueira and Lopes, 2011; Mohaj, 2012). On the other hand, malaria is a dangerous parasitic disease and one of the leading causes of death due to infectious diseases. It is estimated that malaria leads to 500 million infections and 1.1-2.7 millions deaths, annually. Although the disease is familiar to most scientists because of its exotic biology, it is in fact an agent of major global health problem. Malaria affects hundreds of millions of people worldwide and results in significant mortality and devastating social and economic consequences (Kaufman and Rúveda, 2005; Pérez-Picaso, 2009; Nogueira and Lopes, 2011; Mojab, 2012). To some observers, the economic retardation of sub-Saharan Africa can be substantially explained by the prevalence of malaria. In addition to its direct effects on productivity, the presence of this devastating disease scares off foreign investors, traders and tourists (Mihigo, 2005). Moreover, most of the drugs available to treat malaria are decades old and are frequently limited in efficacy, plagued by severe side effects and poor patient compliance, or hamstrung by drug resistance (Mihigo, 2005; Cimanga et al., 2008; Tamura et al., 2010).

Therefore, the availability of effective and affordable drugs remains a central requirement for the management of malaria. Regrettably, the spread of many current drugs resistance through most malaria endemic areas

of the world has degraded the usefulness of these drugs almost completely with serious consequences for malaria treatment and control. The clinical consequences of such resistance are well described in terms of increased morbidity and mortality (Tamura et al., 2010).

Overcoming or reducing/circumventing malaria burden requires the adoption of several strategies, central to these is the use of effective chemotherapy for those who need it. For this reason, it is imperative that new lines of drugs be explored before existing drugs lose too much efficacy (Mihigo, 2005; Tamura et al., 2010; Russell et al., 2013). The challenge ahead lies in determining the best alternative therapies and the establishment of mechanisms to ensure that improved drugs are sustainably discovered and developed into the near future (Mihigo, 2005; Tamura et al., 2010). The isolation of artemisinin, a highly active compound against drug-resistant *Plasmodium falciparum* from *Artemisia annua* has stimulated interest in plants as new anti-malarial sources (Mihigo, 2005; Bero et al., 2009; Kaur et al., 2009). In most countries where this disease occurs, medicinal plants are used in traditional practices and people have found relief that could justify and/or support their use. In this scenario, we hypothesized that Congolese plants used in traditional medicine and claimed to be affective against malaria may be looked at as an important source of anti-plasmodial agents.

Thus, in our continuing efforts to search for bioactive compounds from Congolese plants used in traditional medicine, we carried out the anti-plasmodial investigations of 8 plants used to treat malaria in the Congolese traditional medicine. These are: *Alchornea cordifolia*, *Caloncoba welwitschii*, *Cnestis ferruginea*, *Dacryodes edulis, Hymenocardia acida*, *Manotes pruinosa*, *Nauclea latifolia*, and *Percea americana*.

2. Materials and Methods

Plant materials collection

A total of eight plants, representing seven families and, ethno-medically used by Congolese traditional healers to treat malaria were selected and collected from their natural habitats in Kinshasa, DR Congo. The selection was based on the criterion of being phytochemically less studied. Collected plant parts included the leaves (L), stem bark (SB) and root bark (RB) for each plant species. The collected plant materials were authenticated by Mr Boniface Nlandu of the INERA (Institut National d'Etudes et Recherches Agronomiques) Herbarium located at the Faculty of Science/University of Kinshasa. A voucher specimen of each plant is on deposit at the INERA herbarium. The plant materials were separately dried under shade at room temperature, then ground into a powder that was used for extractions.

Extractions

About 10 g of each plant part were extracted (root temperature maceration and percolation) using dichloromethane (DCM)/methanol (M) (1:1, 24 hrs) followed by M (48 hrs) to give the organic (combined) fractions, and water (24 hrs) to give the aqueous fractions. The extracting solvent was about 15 mL/g. After filtration using appropriate Whatman filter papers and removal of solvent, using a rotary evaporator for the organic fractions or a freeze dryer for the aqueous fractions, 42 extracts were obtained and submitted to anti-plasmodial analyses.

In vitro anti-plasmodial test

All samples (crude extracts) were tested in duplicate on at least one occasion against the chloroquine sensitive (CQS) D10 strain of *P. falciparum* at a standard concentration range of 100 to 0.2 µg/mL. Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained based on reported literature procedures (Tona et al., 1999; Cimanga et al., 2008). Briefly, the test samples were prepared to a 2 mg/mL stock solution in 10 % methanol or 10 % DMSO and were tested as a suspension if not properly dissolved. The test samples were stored at -20 ˚C until use and CQ was used as the reference drug. A full-dose response was performed with a starting concentration of 100 µg/mL, which was serially diluted 2-fold in complete medium to give 10 concentrations; with the lowest concentration being 0.2 µg/mL. CQ was tested at a starting concentration of 100 µg/mL using the same dilution technique. The highest concentration of solvent to which the parasites were exposed to had no measurable effect on the parasite viability (data not shown). The 50 % inhibition concentration (IC_{50}) values were obtained using a non-linear dose-response curve fitting analysis via GraphPad Prism v.4.0 software.

3. Results and Discussion

Forty-two (42) organic [dichloromethane-methanol (1:1)] and aqueous extracts of eight (8) plants commonly used by traditional healers in the Democratic Republic of Congo for the treatment of malaria have been investigated for their *in vitro* anti-plasmodial activities. The results are presented in table format showing the plant species and family, used plant parts, type of extract, and *in vitro* anti-plasmodial activity (IC_{50} values). In general, most of the samples were inactive at the concentration tested. Test samples originating from *Cnestis ferruginea* (CFLO), *Nauclea latifolia* (NLSO),

Dacryodes edulis (DELO, DERO, and DESO), *Alchornea cordifolia* (ACLO and ACLW) and *Caloncoba welwitschii* (CWSO) showed moderate activity with IC_{50} values ranging between 17.5 to 24.0 µg/mL, DESO and ACLW having been found to be the most active extracts (Tab. 1).

Table 1. Anti-plasmodial activity of selected Congolese medicinal plants

Plant species	**Plant part**	**Solvent**	**Plant sample Code[a]**	**Anti-plasmodial activity (µg/mL)**
Alchornea cordifolia (Euphorbiaceae)	L	Organic	ACLO	20.5
		Water	ACLW	17.5
Dacryodes edulis (Burseraceae)	L	Organic	DELO	21.8
		Water	DELW	94.2
	SB	Organic	DESO	17.5
		Water	DESW	26.4
	RB	Organic	DERO	21.7
		Water	DERW	77.5
Nauclea latifolia (Rubiaceae)	L	Organic	NLLO	96.4
		Water	NLLW	> 100
	SB	Organic	NLSO	19.0
		Water	NLSW	> 100
	RB	Organic	NLRO	59.2
		Water	NLRW	> 100
Cnestis ferruginea (Connaraceae)	L	Organic	CFLO	23.6
		Water	CFLW	> 100
	SB	Organic	CFSO	19.6
		Water	CFSW	68.5
	RB	Organic	CFRO	74.3
		Water	CFRW	> 100
Persea americana (Lauraceae)	L	Organic	PALO	52.6
		Water	PALW	> 100
	SB	Organic	PASO	31.6
		Water	PASW	> 100
	RB	Organic	PARO	30.1
		Water	PARW	> 100
Hymenocardia acida (Phyllanthaceae)	L	Organic	HALO	46.0
		Water	HALW	> 100
	SB	Organic	HASO	34.9
		Water	HASW	> 100
	RB	Organic	HARO	76.7
		Water	HARW	91.6
Manotes pruinosa (Connaraceae)	L	Organic	MPLO	70.5
		Water	MPLW	> 100
	SB	Organic	MPSO	41.4
		Water	MPSW	55.5
	RB	Organic	MPRO	73.3
		Water	MPRW	> 100
Caloncoba welwitschii (Flacourtiaceae)	SB	Organic	CWSO	23.8
		Water	CWSW	> 100
	RB	Organic	CWRO	39.9
		Water	CWRW	> 100
Reference (Chloroquine, CQ, ng/mL)				10.0

[a]**Legend**: The first two letters stand for the plant species name. L=leaves, S=stem bark, R=root bark. These are underlined for the case of *A*. *cordifolia* (previous page) for illustration, and apply to all plant species. As for the solvents and plant parts, O=organic extract, W=water (aqueous) extract, and L=leaves, SB=stem bark, and RB=root bark; respectively.

Manotes pruinosa, although well known to be used in traditional medicine and phytochemically not yet extensively worked on, appeared to be the most inactive plant, with the most active plant part having IC_{50} > 55 µg/mL. This might be the raison why not much phytochemical investigation has been reported on this species. The following most inactive plants were *Hymenocardia acida* and *Persea americana* (data shown in table above). Only *A*. *cordifolia* had both organic and aqueous extracts moderately active. Interestingly, this plant species was reported in a previous study to have interesting anti-plasmodial and cytotoxic activities (Mesia et al., 2008). Apart from *A*. *cordifolia*, the organic extracts were, in general, more active than the aqueous ones. We suspect that the variability in data may be due to solubility instead of the solvents used for the extractions. Nevertheless, these plants are perhaps useful in treating malaria-associated symptoms, such as fever, or to enhance the immune system; and might therefore, need to be screened for their anti-pyretic activities. In addition, *Dacryodes edulis, Cnestis ferruginea*, and *Hymenocardia acida* appeared to be among the previously least studied plants. These species, together with the most active *Alchornea cordifolia*, *Caloncoba welwitschii*, *Cnestis ferruginea*, *Dacryodes edulis,* and *Nauclea latifolia* were selected for detailed phytochemical work that could provide a sound basis for the biochemical and pharmacological properties that may be associated with any compounds that could be identified and/or characterized from these plants.

5. Conclusions

In the present study, 8 Congolese plants (*Alchornea cordifolia*, *Caloncoba welwitschii*, *Cnestis ferruginea*, *Dacryodes edulis, Hymenocardia acida*, *Manotes pruinosa*, *Nauclea latifolia*, and *Percea americana*) used in traditional medicine and claimed to cure malaria have been submitted to preliminary anti-plasmodial analyses. The leaves, stem barks, and root barks extracts were evaluated against the chloroquine sensitive (CQS) D10 strain of *P*. *falciparum* at a standard concentration range of 100 to 0.2 µg/mL. In general, most of the samples were inactive at the concentration tested; the most active having shown a moderate activity with IC_{50} values ranging between 17.5 and 24.0 µg/mL. From the present investigation an attempt has been made to highlight the promising plant species for additional studies for anti-malarial drug development. Further studies for the isolation, identification and/or characterisation of individual chemical compounds from the plants under study are envisaged and could lead to significant results.

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