Antiplasmodial activity of some phenolic compounds from Cameroonians Allanblackia

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Abstract

Background: *Plasmodium falciparum*, one of the causative agents of malaria, has high adaptability through mutation and is resistant to many types of anti-malarial drugs. This study presents an *in vitro* assessment of the antiplasmodial activity of some phenolic compounds isolated from plants of the genus *Allanblackia*.

Methods: Tests were performed on well plates filled with a fixed parasitized erythrocytes volume. Compounds to be tested were then added in wells. After incubation, tritiated hypoxanthine is added and the plates were returned to the incubator. After thawing, the nucleic acids are collected. Inhibitory Concentration 50 (IC50) was determined by linear interpolation.

Results: From *Allanblackia floribunda*, have been isolated and characterized 1,7-dihydroxyxanthone 1, macluraxanthone 4, morelloflavone 9, Volkensiflavone 10 and morelloflavone 7-O-glucoside 11; from Allanblackia monticola, α-mangosine 2, rubraxanthone 3, allaxanthone C 5, norcowanine 6 , tovophiline A 7, allaxanthone B 8 and from *Allanblackia gabonensis*, 1,7-dihydroxyxanthone 1. Six of them were evaluated for their antimalarial properties. The most active compound, macluraxanthone, presented a very interesting activity, with an IC50 of 0.36 and 0.27 µg/mL with the F32 and FcM29 strains respectively.

Conclusion: This work confirms that species of *Allanblackia genus* are medicinally important plants containing many biologically active compounds that can be used effectively as antiplasmodial.

Key words: Guttiferaceae, *Allanblackia*, Phenolic compounds, Antiplasmodial activity

DOI: http://dx.doi.org/10.4314/ahs.v15i3.18

Cite as: *Azebaze AGB, Teinkela JEM, Nguemfo EL, Valentin A , Dongmo AB, Vardamides JC. Antiplasmodial activity of some phenolic compounds from Cameroonians Allanblackia. Afri Health Sci. 2015;15(3):835-40. doi: http://dx.doi.org/10.4314/ahs.v15i3.18*

Introduction

Malaria remains one of the major pandemics and is a main public health problem especially in Africa[1]. Malaria is found in tropical and sub-tropical regions of the southern hemisphere. More than two billions people are at risk of contracting this disease worldwide. A microscopic parasite called *Plasmodium falciparum* is responsible for the most dangerous form of malaria. This parasite is transmitted by the female mosquito species belonging to the genus Anopheles[2].

Currently, despite the existing therapeutic arsenal, few drugs are available in the market and are not always accessible to the affected population. Additionally, the increased parasite's resistance to current treatments reinforces the urgent need to search for new antimalarial drugs. Because of the high cost of the prescribed drugs and the various activities displayed by medicinal plants against many diseases, 80% of the world population relies on medicinal plants for their basic and first healthcare. Two current so-called antimalarial drugs have been derived from medicinal plants traditionally used in

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their countries of origin against fevers and malaria. This includes the bark of a tree native to the slopes of the Andean Cordillera (*Cinchona calisaya* and other species of *Cinchona*) and a native Chinese herb *Artemisia annua*[3]. These findings encourage the search for new antimalarial drugs in plant biodiversity. In Cameroon, a large number of plant species have been identified as antimalarial medicinal plants. Pure natural products compounds have been isolated from some of these plants and their antimalarial activities were comparable to or more active than chloroquine on sensitive and resistant strains of *P. falciparum*[4]. Plasmodium falciparum, one of the causative agents of malaria, has high adaptability through mutation and is resistant to many types of anti-malarial drugs. This resistance is a serious setback to antimalarial programs since it precludes the use of cheap and previously effective drugs like chloroquine. New families of active compounds are needed, especially from natural sources in order to decrease the risk of resistance. An alternative solution in many endemic countries is the use of traditional medicinal plants since many of the available antimalarial drugs are from plants sources, and the potential of plants to produce new antiprotozoal agents are considerable.

The phytochemical and pharmacology studies of plants of the genus *Allanblackia* revealed the presence of biologically active secondary metabolites belonging to benzophenones, xanthones, triterpenes, phytosterols and biflavonoids[5-9]. *A floribunda* was studied for antioxidant, antitumor and antimicrobial activities[10], ejaculatory activities[11], antioxidative properties and hypolipidemic effects[12], free radical scavenging activities[13]; *A monticola* for leishmanicidal and cholinesterase activities[14], anti-inflammatory and anti-nociceptive activities[15], anti-oxidative and anti-inflammatory activities[16], apoptotic and antiproliferative activities[9] and for antiplasmodial activity[17,18]; *A. gabonensis* for antiparasitic and antimicrobia[18], hepatho-nephroprotective and antioxidant activities[19], analgesic and anti-inflammatory activities[20].
In the present study, we report on the antiplasmodial activity of isolated phenolic compounds which were not evaluated before as well as the crude extracts from some cameroonian plants of the genus *Allanblackia*: *A. floribunda*, A. monticola and A. gabonensis.

Materials and methods

Plant materials

Allanblackia floribunda, Allanblackia gabonensis and *Allanblackia monticola* plants belonging to the family of Guttiferaceae were collected respectively on top of Mount Kala in the Central Region Cameroon for the first two species and Bangangté area of Western Cameroon to the latter species. They were then identified by Dr. Zapfack from the Botany Department at the University of Yaounde I. Voucher specimens were deposited at the National Herbarium of Cameroon.

Extraction and isolation

Plants were then cut, air-dried and powdered. The powders obtained were then macerated with CH_2Cl_2/MeOH (1/1) at room temperature for 48 h followed by pure methanol for 4 hours. After evaporation under reduced pressure, the crude extracts were obtained[8,10,16]. The various crude extracts were submitted to fractionation using hexane-ethyl acetate of increasing polarity as eluent. Fractions of 300 mL were collected and pooled on the basis of their thin layer chromatography (TLC) profiles. Further purification through successive column chromatography yielded several pure compounds belonging to many classes of compounds.

Identification of compounds

The isolated compounds were then characterized using various spectroscopic and spectrometric techniques such as 1D- and 2D-NMR and MS. Melting points were determined using a Kofler bench and are uncorrected. The mass spectra were recorded on a API Q-STAR PULSAR spectrometer. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker 300 and 75 MHz spectrometer respectively with TMS as internal standard. Coupling constants are expressed in Hertz. NOESY, HMBC, HSQC and Jmod experiments were performed with conventional pulse sequences and on a 400 MHz Brucker spectrometer. Column chromatography (CC) and TLC were carried out on silica gel 60H Merk, 70-230, 200-300 mesh; GGo, GF254 aluminum plates 20 x 20 cm Merck and Analtech; respectively. Spots were visualized by UV lamp (254 nm and 365 nm) or by spraying with 50% H_2SO_4/H_2O solution, or using iodine. The in vitro antimalarial activity was performed on two reference strains of *Plasmodium falciparum*: the F_{32} strain from Tanzania, chloroquine-sensitive and native of-Cameroon FcM_{29} strain resistant to chloroquine[21]. The tests were performed using the radio-isotope technique based on the method previously described by Desjardins[22] and modified by Benoit[23].

Assays of bio-activity

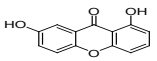
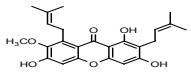
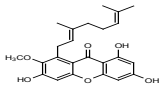
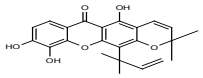
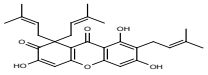
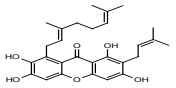
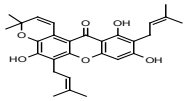
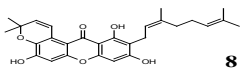
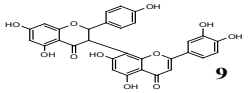
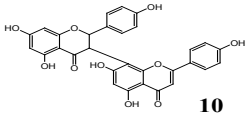
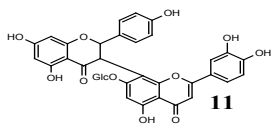
Tests were performed on 96 well plates filled with a fixed parasitized erythrocytes (parasitemia of 1.0% and 1.5% hematocrit) volume. Fractions to be tested (at

different concentrations) were then added in triplicate wells. After 24 h incubation at 37°C, 0.25 μCi of the tritiated hypoxanthine is added in each well (Perkin Elmer 1 μCi/mL, France) and the plates were returned to the incubator for 24 h. At the end of the cycle, the plates are frozen at – 20°C to cause hemolysis of erythrocytes. After thawing (4th day), the nucleic acids are collected onto filters using an automated cell collector (Perkin Elmer, France). The radioactivity on the filter (dried in a microwave and wrapped in plastic to which are added 4 mL of liquid scintillation (Betaplate Scint, Perkin Elmer) is then measured by a β counter (Microbeta Trilux, Perkin Elmer). Inhibitory Concentration 50 (IC50) (sample concentration which inhibits 50% of the parasite growth) is determined by linear interpolation, with the ratio of the percentage of parasitemia on the logarithm of the concentrations of samples. The radioactivity reading determines (by linear regression analysis) the Inhibitory Concentration 50.

Results

After the characterization of the isolated compounds, we identified 05 pure compounds from *A. floribunda:* 1,7-dihydroxyxanthone, macluraxanthone, morelloflavone, volkensiflavone and morelloflavone 7-O-glucoside[10,24], 06 from *A. monticola:* α-mangosine, rubraxanthone, allaxanthone, norcowanine, tovophiline A, allaxanthone B7,[16] and 01 from *A. gabonensis,* 1,7-dihydroxyxanthone[8]. The structures are showed in Table 1.

Table 1. Chemical structure of isolated compounds from *Allanblackia*

Name	Structure	Source	References
1,7-dihydroxyxanthone	 **1**	*A. gabonensis, A. floribunda*	[8,10,16]
α-mangosine	 **2**	*A. monticola*	[8,16]
rubraxanthone	 **3**	*A. monticola*	[8,16]
macluraxanthone	 **4**	*A. floribunda*	[10,21]
allaxanthone C	 **5**	*A. monticola*	[8,16]
norcowanine	 **6**	*A. monticola*	[8,16]
tovophiline A	 **7**	*A. monticola*	[8,16]
allaxanthone B	 **8**	*A. monticola*	[8,16]
morelloflavone	 **9**	*A. floribunda*	[10,24]
volkensiflavone	 **10**	*A. floribunda*	[10,24]
morelloflavone 7-*O*-glucoside	 **11**	*A. floribunda*	[10,24]

It is clear from this table that the phenolic compounds contained in the plants of the genus *Allanblackia* are mainly xanthones and biflavonoids.

Xanthones: The xanthones isolated from *A. floribunda*, are both prenylated and non-prenylated, while those of *A. monticola* have in their structure at least one prenyl or geranyl group. Xanthones from *A. monticola* have a B ring dioxygenated in position 6, 7 and prenyles or geranyles groups are in positions 2, 4, 5 or 8. The isolated compound from *A. gabonensis* is a oxygenated xanthone.

Biflavonoids: These groups of compounds have mainly been isolated from *A. floribunda* and their two constitutive units are different (flavanone-flavone type).
The linear regression allowed determining the IC50 of the tested compounds (Table 2).

Table 2. Evaluation of *in vitro* antiplasmodial activity of the different compounds tested on *P. falciparum* F_{32} and FcM_{29} strains in comparison with chloroquine as reference (IC_{50} in µg/mL)

Compounds		F_{32}/24h	F_{32}/72h	FcM_{29}/24h	FcM_{29}/72H
		IC_{50}	IC_{50}	IC_{50}	IC_{50}
1,7-dihydroxy xanthone	**1**	16.05 +/- 9.02	19.87 +/- 19.15	17.93 +/- 5.40	15.34 +/- 4.23
macluraxanthone	**4**	0.46 +/- 0.12	0.36 +/- 0.06	0.33 +/- 0.08	0.27 +/- 0.01
allaxanthone B	**8**	3.70 +/- 1.05	3.09 +/- 0.13	3.93 +/- 0.40	3.43 +/- 0.57
morreloflavone	**9**	11.77 +/- 9.55	3.36 +/- 2.00	12.59 +/- 12.03	4.80 +/- 2.21
volkensiflavone	**10**	0.99 +/- 0.62	1.18 +/- 1.25	0.93 +/- 0.20	0.95 +/- 0.27
morreloflavone 7-*O*-glucoside	**11**	11.45 +/- 14.51	8.38 +/- 10.87	28.92 +/- 7.45	23.82 +/- 7.58
chloroquine		0.036	0.036	0.57	0.57
EAF		5.38 +/- 0.83	5.43 +/- 1.31	4.92 +/- 1.43	4.48 +/- 1.47
EAG		35.38 +/- 2.87	32.01 +/- 1.21	25.17 +/- 2.87	24.30 +/- 4.04
EAM		3.27 +/- 0.25	3.33 +/- 0.17	3.08 +/- 0.08	2.13 +/- 1.07

EAF: Crude extract of the bark of *A. floribunda*, EAM: Crude extract from the bark of *A. monticola*, EAG: Crude extract fromthe bark of *A. gabonensis*.

After 24h of contact with the parasite, volkensiflavone (IC50: 0.99 µg/mL) and macluraxanthone (IC50: 0.46 µg/mL) displayed the best activity on the F_{32} strain while chloroquine (IC50: 0.036 µg/mL) was used as reference. With FcM29 strain, volkensiflavone (IC50: 0.93 µg/mL) and macluraxanthone (IC50: 0.33 µg/mL) remained the most active compounds, but the macluraxanthone was more active than the reference (chloroquine: IC50: 0.57 µg/mL). After 72 h of contact, macluraxanthone (IC50: 0.36 µg/mL) exhibited the high activity on the F_{32} strain and with FcM_{29} strain, it was most active (IC50: 0.27 µg/mL). Additionally, several other compounds also showed good activities: volkensiflavone (IC50: 0.95 µg/mL) and α-mangosine

(IC50: 0.33 µg/mL). For the most active compounds (macluraxanthone), we noted that when it was allowed to go from 24 to 72 h of contact with parasite, there was increased activity of 0.1 µg/mL with the F_{32} strain and 0.06 µg/mL with FcM29 strain. Similarly, for α-mangosine, we got an increase in activity of 1.39 µg/mL with FcM_{29} layer. In contrast, for volkensiflavone there was a decrease in activity of 0.19 µg/mL with the F_{32} strain and 0.02 µg/mL with FcM_{29} strain when going from 24 to 72 h of contact with parasite.

Discussion

From all tested compounds, macluraxanthone is the most active compound on two strains of *Plasmodium* with a mean IC50 of 0.36 and 0.27 µg/mL for the F_{32} and FcM_{29} strains respectively. Five of those prenylated xanthones (α-mangosine, tovophiline A, allaxanthone C, rubraxanthone, norcowanine) isolated form *A. monticola* previously tested for antiplasmodial properties had displayed after 24 h of contact with the parasite a significant antiplasmodial activity (IC50: 1.96 - 3,16 µg/mL) on the F_{32} strain and (IC50: 1.72 - 3.22 µg/mL) on FcM_{29}[17,18]. This activity is less interesting than that of macluraxanthone (IC50: 0.46 and 0.33 µg/mL respectively with F_{32} and FcM_{29}). We also noted that its chemical structure contains two isoprene chain groups.1,7 dihydroxanthone (without isoprenyl group) showed very low activity (16,05 µg/mL).

It can then be suggested that the antiplasmodial activities of these compounds could be improved by the presence of isoprenyl groups on their structures. For comparison, four xanthones and three of their analogues have been isolated from *Cratoxylum maingayi* and *Cratoxylum cochinchinense* (Clusiaceae), respectively, and these compounds showed antiplasmodial activity against *P. falciparum* at concentrations of 11.0 to 1.9 µM[25], less active than macluraxanthone (0.6 µM). As for biflavonoids, those flavanone-flavone types are the most active. Volkensiflavone exhibited antiplasmodial activity with mean IC50 of 1.5 µM, which is a significant value compared to those of other biflavonoids. For example, four biflavonoids were isolated from *Ormocarpum kirkii* and showed antiplasmodial activity toward *P. falciparum* strain K1; isochamaejasmin was the most active among the four with an IC50 of 7.3 µM[26]. For the three biflavonoides tested, we realize that the presence of the hydroxyl group in position 3' in the morelloflavone and morelloflavone 7-O-glucoside (IC50: 11.77 and 11.45 µg/mL respectively) causes a sharp drop in activity compared to that of the volkensiflavone (IC50: 0.99 µg/mL) with the F_{32} strain after 24 h contact with the parasite, but also in general way. Moreover, it was reported that the activity exhibited by the crude extract of *A. monticola* was ascribed to the synergy of its polyisoprenylated xanthones.

Conclusion

This work confirms that species of *Allanblackia genus* are medicinally important plants containing many biologically active compounds that can be used effectively as antiplasmodial. The antiplasmodial activity of these isolated phenolic compounds were particularly high and more so with isoprenylated ones. This study can validate at least in part the uses of species of *Allanblackia* for the treatment of febrile aches. Further studies are however required to establish the role played by the isoprenylated groups in antiplasmodial study. The isolated compounds tested in this investigation can also be suggested as lead compounds for future development of economic antimalarial drugs.

Competing interests

The authors declare that they have no competing interest.

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