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Full Length Research Paper

In vitro anti-malarial activity of 20 quinones isolated from four plants used by traditional healers in the Democratic Republic of Congo

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The anti-malarial activity of 20 quinones isolated from *Cassia alata*, *Cassia occidentalis*, *Garcinia kola* and *Ocimum basilicum* was investigated *in vitro* using the micro dilution test of Desjardin by a visual evaluation on thin blood smears. The six quinones isolated from *C. occidentalis*, three from *C. alata* and three from *O. basilicum* were found to be the most active with an IC_{50} value of below 1 µg/ml. The others quinones showed a moderate activity with IC_{50} values of between 5 and 20 µg/ml.

Key words: *Cassia alata*, *Cassia occidentalis, Garcinia kola, Ocimum basilicum,* quinones, anti-malarial activity, *in vitro.*

INTRODUCTION

Malaria accounts for 1 - 3 million deaths yearly worldwide, with most of this burden occurring in children under 5 years of age in sub - Saharan Africa (Breman 2001). In Kinshasa, Democratic Republic of the Congo, malaria is prevalent in pupils in suburbs where most of poor people live (Kazadi et al., 2004). An ethno botanical survey carried out in these areas had shown that more than 70% of populations use plants to treat fever (supposed to be malaria) (Ngalamulume et al., 1995). 58 species of plants and recipes commonly used were identified. Among those, *Cassia alata, Cassia occidentalis, Garcinia. kola and Ocimum basillicum* were the most frequently used (Kasuku et al., 1999).

The intolerable burden of malaria can be conquered by, among other things, searching new molecules with potent anti - malarial activity in plants which always have been the most common sources of medicines.

The aim of the present work is to evaluate *in vitro* anti - malarial activities of 20 quinones isolated from these four plants: *C. alata* (4), *C. occidentalis* (6), *G. kola* seeds (5) and *O. basillicum* (5). In the chemotherapy of malaria, most molecules belong to the class of alkaloids (Federici et al., 2000; Andrade – Neto et al., 2003; Zirihi et al., 2005) and terpenes (Li et al., 1992). The class of quinones has few representative such as Atovaquone (Fowler et al., 1994; Basco et al., 1995), Lapachol and Lapinone (The Merck Index 2005). Quinones as antimalarial are attracting attention recently (Kanokmedhakul et al., 2005; Laurent et al., 2006; Eyong et al., 2006) and the present investigation is in line with this interest.

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MATERIALS AND METHODS

Plant materials

Plants were collected in Kinshasa/ Kisenso, Democratic Republic of Congo and were authenticated by the Herbarium service of Department of Biology, University of Kinshasa where voucher specimens are preserved. The leaves and seeds were air dried at room temperature for 20 days and then grinded with pestle and mortar.

Preparation of crude ethanol extract

Crude ethanol extracts were prepared for primary *in vitro* anti-malarial assay. The dried and grinded leaves or seeds (20 g) were extracted with ethanol (2 ×1 L) at room temperature. After the removal of ethanol *in vacuo*, the crude extracts obtained were submitted for anti-malarial test in *vitro.*

Table 1. *In vitro* antimalarial activity of crude ethanol extracts.

Plant	Concentration (µg/ml)	Growth inhibition %
Cassia alata	25	83
	12.5	53
Cassia occidentalis	25	93
	12.5	24
Garcinia kola	n.d	n.d
Ocimum basilicum	12.5	78
Quinine	0.1	100

Extraction of quinones

The dried and grinded leaves (200 g) were extracted with methanol (2 x 2.5 l) at room temperature. The methanol extract was suspended in a Methanol - H_2O 7/3 mixture and then extracted successively with Petroleum ether (60 - 80°C) and $CHCl_3$.The $CHCl_3$ fraction was subjected to vacuum liquid chromatography (VLC) on silica gel previously soaked in a 0.5N oxalic acid solution and dried over night. The fractions having the same TLC profile were collected, concentrated together and then submitted each to column chromatography on silica gel using the following solvent systems: Pyridine/Amyl alcohol/H_2O 4 : 6 : 3 for *C. alata* quinones; $CHCl_3$/Ethyl Acetate 5 :1 for *C. occidentalis*; Ethyl Acetate/ Petroleum Ether 4 :1.2 for *G. kola* and *O. basillicum* quinones. Four fractions (CA_1 - CA_4) for *C. alata*, six fractions (CO_1 - CO_6) for *C. occidentalis*, five fractions (GK_1 - GK_5) for *G. kola* and five fractions (OB_1 - OB_5) for *O. basillicum* were obtained. All these fractions showed a positive Borntrager test with a 5% KOH alcoholic solution.

Anti - malarial activity

The anti - malarial activities of quinones were evaluated *in vitro* on their ability to inhibit *Plasmodium falciparum* growth on RPMI 1640 medium. This method estimates the growth of parasites incubated in RPMI 1640 medium containing plants extracts in various concentrations (Desjardins et al., 1979). Parasitemia was determined on blood smears 48 h after extracts and parasites contact (Benoit et al., 1996). The IC_{50} values were determined graphically in terms of concentration versus inhibition percentage.

RESULTS AND DISCUSSION

Ethanol extracts

Screening the anti - malarial activity of crude extracts is a first step in the isolation of new molecules with potent activity (Ma, Zhang and al. 2006; Njoroge G.N. and al.2006). Anti - malarial activities results of crude ethanol extracts (Table 1) show that for a concentration of 25 µg/ml, *C. occidentalis* extract is the most active with 93% of plasmodium growth inhibition compared to the others crude extracts. *C. occidentalis* extracts have been reported to possess various biological activities (Sharma et al., 2000; Bin-Hafeez et al., 2001). The anti – malarial activities of *C. occidentalis* and *G. kola* have been reported by Tona et al. (2001). It is likely that these activities could be attributed to the presence of quinones in these plants. The Petroleum Ether and ethanol extracts of *C. alata* are reported to have a potent inhibitory activity against *Chrysomya megacephale* (Kumarasinghe et al., 2002) and against opportunistic HIV patients' infections (Crockett et al., 1992). *Ocimum* genus species are known to have antimicrobial, antispasmodic and mosquitoes repellent effects (Fatope he results obtained for the 20 quinones are summarized in (Table 2). 12 quinones (CO_1 - CO_6; CA_1, CA_2 and CA_4; OB_1, OB_2 and OB_4) were found to be the most active with the IC_{50} values below 1 µg/ml. 4 quinones showed an acceptable activity with the IC_{50} values between 1 and 5.5 µ/ml. The others have shown a low activity (IC_{50} > 10 µg/ml).

Natural quinones have benzoquinone, naphtoquinone or anthraquinone structures. A growing interest is granted to these compounds from the description of the antimalarial activity of Atovaquone (Basco et al., 1995). Three quinones isolated from *Salacia krausii* presented very high activity on *P. falciparum* (Figueiredo et al., 1998). Weiss et al. reported a good *in vitro* antimalarial activity for Naphthoquinones isolated from *Kigelia pinnata* with IC_{50} values around 0.002 µg/ml (Weiss et al., 2000). This naphtoquinone is more active than all isolated by us. A new Xestoquinone isolated in Vanuatu from a marine sponge (*Xestospongia sp.*) exhibited also an interesting inhibitory activity on plasmodium growth with an IC_{50} value of 3 µM (Laurent et al., 2006). These results added to ours, showed that quinones are compounds with an interesting antimalarial activity. They could be used as starting point for the synthesis of molecules much more active such as the reported synthesis of an aminonaphtoquinone which has shown a IC_{50} of 37,3 ng/ml (Kapadia et al., 2001).

Conclusion

Quinones isolated from *C. alata, C. occidentalis, G. kola and O. basilicum* have interesting antimalarial activities (IC_{50} < 1 µg/ml for 12 of them). Further investigations are required in order to characterize the isolated compounds.

Table 2. *In vitro* IC_{50} (µg/ml) of isolated quinones against *Plasmodium falciparum.*

Plant	Isolated quinone	Rf	IC_{50} (µg/ml)
Cassia alata (leaves)	CA_1	0.61	<0.1
	CA_2	0.70	5.4
	CA_3	0.86	0.54
	CA_4	0.92	<0.25
Cassia occidentalis (leaves)	CO_1	0.17	0.25
	CO_2	0.28	0.25
	CO_3	0.33	<1
	CO_4	0.53	0.25
	CO_5	0.64	<0.1
	CO_6	0.84	<0.1
Garcinia kola (seeds)	GK_1	0.17	1.02
	GK_2	0.25	2.0
	GK_3	0.42	12.9
	GK_4	0.54	15.75
	GK_5	0.85	n.d
Ocimum basilicum (leaves)	OB_1	0.12	0.52
	OB_2	0.23	<0.35
	OB_3	0.50	1.42
	OB_4	0.63	<0.35
	OB_5	0.88	18
Naphthoquinones (*Kigelia pinnata)**			0.002
Aminonaphtoquinones**			37.3 ng/ml**
Chloroquine			72 ng/ml**

*Weiss et al. (2000).
**Reported by Kapadia et al. (2001).

REFERENCES

Andrade-Neto VF, Brandao MGL, Stehmann JA, Oliveira LA, Krettli AU (2003). Antimalarial Activity of Cinchona-like species plants used to treat fever and malaria in Brazil. J. Ethnopharmacol. 87(2-3): 253-256.

Basco LK, Ramiliarisoa O, Le Bras J, (1995). *In vitro* activity of atovaquone against the African Isolates and clones of *Plasmodium falciparum.* Am. J. Trop. Med. 53(4): 388-391.

Benoît F, Valentin A, Pellisier Y, Diafouka F, Marion C, Kone – Bamba D, Kone M, Yapo A, Bastide JM (1996). *In vitro* antimalarial activity of vegetal extracts used in West African Traditional Medicine. Am. J. Trop. Med. Hyg. 54(1): 67-71.

Bin-Hafeez B, Ahmad I, Haque R, Raisuddin S (2001). Protective effect of *Cassia occidentalis* L. on Cyclophosphamide-induced suppression of humoral immunity in mice. J. Ethnopharmacol. 75(1): 13-18.

Breman JG (2001). The ears of the hippopotamus: manifestations, determinants and estimates of the Malaria burden. Am. J. Trop. Med. Hyg. 64(Suppl. 1-2): 1– 11.

Crockett CO, Guede-Guina F, Pugh D, Vangah-Manda M, Robinson TJ, Olubadewo JO, Ochillo RF (1992). *Cassia alata* and the preclinical search for therapeutic agents for The treatment of opportunistic infections in AIDS patients. Cell. Mol. Biol. 38(7): 799-802.

Eyong KO, Folefoc GN, Kuete V, Beng P, Krohn K, Hussain H, Nkengfack AE, Saeftel M, Sarite SR, Hoerauf A (2006). A naphthoquinone-anthraquinone ether coupled pigment, as a potential antimicrobial and antimalarial agent from *Newbouldia laevis*. Phytochemistry 67(6): 605-609.

Eyong KO, Folefoc GN, Kuete V, Beng VP, Krohn K, Hussain H, Nkengfack AE, Saeftel M, Sarite SR, Hoerauf A (2006). A naphthoquinone-anthraquinone ether coupled pigment, as a potential antimicrobial and antimalarial agent from *Newbouldia laevis.*
Phytochemistry 67(6): 605-609.

Fatope MO, Takeda Y (1988). The constituents of the leaves of *Ocimum basilicum*. Planta. Med. 542: 190-191.

Federicci E, Palazzino G, Nicoletti M, Caleffi C (2000). Antiplasmodial activity of the alkaloids of *Paschiera fuchsiaefolia*. Planta. Med. 66(1): 93-95.

Figueiredo JN, Raz B, Sequin U (1998). Novel quinone methides from *Salacia kraussii* with *in vitro* Antimalarial activity. J. Nat. Prod. 61(6): 718-723.

Fowler RE, Billingsley PF, Pudney M, Sinden RE (1994). Inhibitory action of the antimalarial compound atovaquone (566C80) against *Plasmodium berghei* ANKA in mosquito, Anopheles stphensi. Parasitology 108(Part 4): 383-388.

Kanockmedhakul M, Kanockmedhakul S, Phatchana R (2005). Biological activity of Anthraquinones and Triterpenoids from *Prismatomeris fragrans*. J. Ethnopharmacol. 100(3): 284-288.

Kapadia GJ, Azuine MA, Balasubramanian V, Sridhar R (2001). Amininoquinones: a novel class of compounds with potent antimalarial activity against Plasmodium falciparum; Pharmacol. Res.

43(4): 363-367.
Kazadi W, Sexton JD, Bigoma M, W'Okanga B, Way M (2004). Malaria in primary school Children and infants in Kinshasa. Democratic Republic of Congo. Am. J. Trop. Med. Hyg. 71(Suppl. 2): 97-102.
Kumarasinghe SP, Karunaweera ND, Ihalamulla RL, Arambewela LS, Dissanayake RD (2002). Larvicidal effects of mineral turpentine, low aromatic white spirits, aqueous extracts of *Cassia alata*, and aqueous extracts, ethanolic extracts and essential oil of betel leaf (Piper betel) on *Chrysomya megacephala.* Int. J. Dermatol. 41(12): 877-880.
Laurent D, Jullian V, Parenty A, Knibiehler M, Dorin D, Schmitt S, Lozach O, Lebouvier N, Frostin M, Alby F, Maura S, Doerig C, Meijer L, Savant M (2006). Antimalarial potential of xestoquinone, a protein kinase inhibitor isolated from a Vanuatu marine Sponge *Xestospongia sp*. Bioorg. Med. Chem. 14(13): 4477-4482.
Li X, Rieckmann K (1992). A bioassay for derivatives of Qinghaosu (artemisinin) Trop. Med. Parasitol. 43: 195-196.
Ma C, Zhang HJ, Tan GT, Hung NV, Cuong NM, Soejarto DD, Fong HH (2006). Antimalarial compounds from *Grewia bilamellata* J. Nat. Prod. 69(3): 346-350.
Njoroge GN, Bussmann RW (2006). Diversity and utilization of antimalarial ethnophytotherapeutic Remedies among the Kikuyus (Central Kenya). J. Ethnobiol. Ethnomedicine 2: 8.
Sharma N, Trikha P, Athar M, Raisuddin S (2000). *In vitro* inhibition of carcinogen induced mutagenicity by *Cassia* occidentalis and *Emblica officinalis*. Drug Chem. Toxicol. 23(3): 477-484.
Skaltsa H, Philianos S (1986). Chemical study of *Ocimum basilicum* L. Part 1: Plant. Med. Phytother. 20(4): 291-299.
The Merck Index: An Encyclopaedia of Chemicals and Drugs.13th Edition Merck and Co., Inc Rahway. N.J. USA. (2005).
Tona L, Mesia K, Ngimbi NP, Chrimwami B, Okond'ahoka, Cimanga K, de Bruyne T, Apers S, Hermans N, Totte J, Pieters L, Vlietinck AJ (2001). *In-vivo* antimalarial activity of *Cassia occidentalis*, *Morinda morindoides* and *Phyllanthus niruri.* Ann. Trop. Med. Parasitol. 95(1): 47-57.
Weiss CR, Moideen SV, Croft SL, Hougton PJ (2000). Activity of extracts and isolated Naphthoquinones from *Kigelia pinnata* against *Plasmodium falciparum.* J. Nat. Prod. 63(9): 1306-1309.
Zihiri GN, Grellier P, Guede – Gina F, Bodo B, Mambu L (2005). Isolation, characterization and antiplasmodial activity of steroidal alkaloids from *Funtumia elastica* (Preuss) Stapf. Bioorg. Med. Chem. Lett. 16(10): 2637- 2640.