

Anti-plasmodial activity of *Ailanthus excelsa* [☆]

Mario Dell'Agli ^{a,*}, Germana V. Galli ^a, Silvia Parapini ^b, Nicoletta Basilico ^b,
Donatella Taramelli ^b, Ataa Said ^c, Khaled Rashed ^c, Enrica Bosisio ^a

^a Department of Pharmacological Sciences, University of Milan, Milan, Italy

^b Department of Public Health, Microbiology and Virology, University of Milan, Milan, Italy

^c National Research Centre, Pharmacognosy Department, Dokki, Giza, Egypt

Received 25 January 2007; accepted 5 November 2007

Available online 31 December 2007

Abstract

The anti-plasmodial activity of *Ailanthus excelsa* stem bark was investigated. The methanolic extract inhibited *in vitro* growth of chloroquine-sensitive (D10) and resistant strains (W2) of *Plasmodium falciparum* (IC₅₀ 4.6 and 2.8 µg/ml, respectively). The effect was retained in the chloroform fraction (3.1 and 2.1 µg/ml, respectively). The anti-plasmodial activity could be ascribed to the impairment of haemoglobin degradation through the inhibition of plasmepsin II activity (IC₅₀ of 13.43 ± 1.74 µg/ml) and of the haem detoxification to haemozoin.

© 2007 Elsevier B.V. All rights reserved.

Keywords: *Ailanthus excelsa*; Malaria; Anti-plasmodial activity; Plasmepsins; Haemozoin

1. Introduction

Ailanthus excelsa is a fast-growing and large tree, up to 25 m tall, commonly known as Indian Tree of Heaven. In traditional medicine the bark is being used as anti-spasmodic and expectorant [1]. *A. excelsa* has been shown to possess anti-bacterial [2], anti-leukemic [3] and anti-fungal activities [4]. As for the other *Ailanthus* species *A. excelsa* contains the bitter principles known as quassinoids. Quassinoids isolated from *Ailanthus* spp. possess several biological activities including anti-tumor [5], anti-viral [6,7], anti-tuberculosis [8] properties reported in a Review [9]. Quassinoids were also shown to be the active principles responsible for the anti-malarial activity of *Ailanthus altissima*. The chloroform extract obtained from the stem and root bark of this plant inhibited *in vitro* the growth of *Plasmodium falciparum* and was active against *P. berghei* infections in mice [10]. The anti-plasmodial activity has been mainly referred to the presence of ailanthon, a quassinoid responsible for the anti-malarial activity recently observed also for the seedlings of *A. altissima* [11].

[☆] Mario Dell'Agli and Germana V. Galli contributed equally to this paper.

* Corresponding author. Tel.: +44 02 5031 8345; fax: +44 02 5031 8391.

E-mail address: mario.dellagli@unimi.it (M. Dell'Agli).

Malaria represents one of the key priorities for the WHO due to the unavailability of a vaccine and the spread and intensification of drug resistance over the last 15–20 years.

Modern pharmaceuticals are unavailable and/or unaffordable for the populations living in those areas affected by malaria. In such conditions non-industrialized countries rely on traditional medicine and locally grown plants for the cure of malaria. Research Initiative on Traditional Antimalarial Methods (RITAM) is promoting the use of traditional remedies based on the concept of evidence-based medicine. While *A. altissima* and other plants of the Simaroubaceae have been investigated for their efficacy as anti-malarials [12], *A. excelsa*, which is used as anti-pyretic [1], was never explored at this regard. The present work was undertaken to evaluate *in vitro* the anti-plasmodial activity of extracts from *A. excelsa* and to investigate the mechanism of action. At this aim the extracts were assayed on chloroquine-sensitive and resistant strains of *P. falciparum* at the intra-erythrocytic stage. The survival of malaria parasites in the host cells relies on the degradation of haemoglobin in the food vacuole with the involvement of several proteinases, including plasmepsins (PLM), a family of aspartyl proteinases specific to *Pf*. The inhibition of PLM leads to the starvation of the parasite [13], then it represents a feasible mechanism of action for the anti-plasmodial effect. In addition, once haemoglobin is degraded, the parasite detoxifies the excess of released haem by converting it into the crystalline pigment haemozoin [14]. A synthetic compound, called β -haematin spectroscopically identical to the native pigment can be obtained *in vitro*. Quinoline anti-malarials have been shown to inhibit β -haematin formation [15]. Then we evaluated the ability of *A. excelsa* to inhibit PLM II and IV and the formation of β -haematin *in vitro*.

2. Experimental

2.1. Plant material

A. excelsa Roxb (Simaroubaceae), stem bark collected from Zoo garden, Giza, Egypt, in April 2002, was identified by Prof. Dr. Kamal El Batany, Professor of Taxonomy and Botany, Faculty of Science, Cairo University. A voucher was deposited in National Research Centre Herbarium.

2.2. Extraction

Dried powdered stem bark (1 kg) was Soxhlet-extracted with petroleum ether, Et₂O, CHCl₃, and MeOH (70%) and concentrated *in vacuo*. MeOH extract (67.5 g) was added with 500 ml of distilled water and extracted with CHCl₃. The CHCl₃ solution was shaken with 10% HCl. The acid layer was made alkaline with NH₄OH to pH 9, then it was extracted with CHCl₃. The CHCl₃ layer dried was evaporated giving 1.7 g of crude alkaloid chromatographed over silica gel column using CHCl₃ and increasing polarity with MeOH. Elution with chloroform gave canthin-6-one identified by comparison with the reported spectral data [16,17].

2.3. Drug susceptibility assay on *P. falciparum*

The CQ-sensitive (D10) and the CQ-resistant (W2) strains of *P. falciparum* were sustained *in vitro* as described by Trager and Jensen [18]. Parasites were maintained at 5% hematocrit (human type A-positive red blood cells) in RPMI 1640 (Gibco BRL, NaHCO₃ 24 mM) medium with the addition of 10% heat-inactivated A-positive human plasma, 20 mM Hepes (Euroclone), 2 mM glutamine (Euroclone). Compounds were dissolved in either water (chloroquine) or DMSO and then diluted with the medium to achieve the required concentrations (final DMSO concentration <1%). Asynchronous cultures with parasitemia of 1–1.5% and 1% final hematocrit were aliquoted into the plates and incubated for 72 h at 37 °C. Parasite growth was determined spectrophotometrically (OD₆₅₀) by measuring the activity of the parasite lactate dehydrogenase (LDH), according to a modified version of Makler's method in control and drug-treated cultures [19]. Anti-plasmodial activity is expressed as the 50% inhibitory concentrations (IC₅₀), each IC₅₀ value is the mean \pm SD of at least three separate experiments performed in duplicate.

2.4. PLMs inhibition assays

Recombinant pro-PLM II and IV were purified from BL21-(DE3) pLysS *E. coli* (Invitrogen, Milano) according to Hill J. et al. [20], with slight modifications [21]. Protein concentration was determined according to the method of

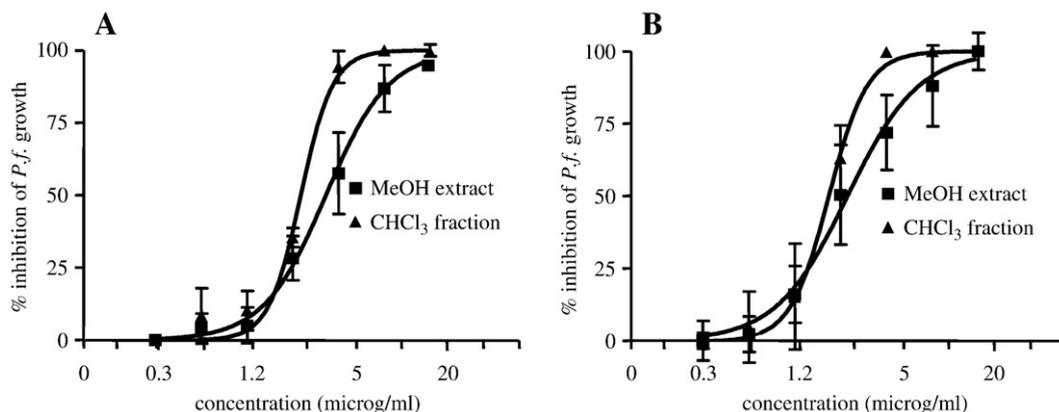


Fig. 1. Anti-plasmodial activity of MeOH and CHCl₃ extracts against *P. falciparum* D10 (A) and W2 (B) strains.

Bradford [22]. Proteins were diluted to the final concentration of 0.5 mg/ml in 50% glycerol and stored at -20°C . Pro-PLM II and IV were activated by addition of one tenth volume of 100 mM sodium acetate buffer pH 4.7 by incubation at 37°C for 90 min. The enzyme activity of PLM II and IV was evaluated spectrophotometrically at 300 nm as described [20].

The CHCl₃ fraction from *A. excelsa* dissolved in DMSO was tested at 5–50 $\mu\text{g/ml}$ (final solvent concentration 1% of the sample volume). PS777621 [23] was used as the reference compound. The assays were conducted twice in triplicate. Inhibition curves and IC₅₀ values were calculated by a non-linear regression for sigmoidal curves using Graph Pad Prism 4.

2.5. β -Haematin inhibitory assay

β -Haematin formation was assayed by a spectrophotometric microassay BHIA (β -Haematin inhibitory assay) previously reported [24]. Haemin dissolved in DMSO (0.4 $\mu\text{mol/well}$) was distributed in 96-well microplates. CHCl₃ fraction dissolved in DMSO was added in doses ranging from 0.3 to 5 mg/ml. Chloroquine was used as reference compound and showed an IC₅₀ of 0.9 ± 0.05 mg/ml (mean \pm SD).

2.6. Cytotoxicity assay

Cytotoxicity was evaluated in human fibroblasts from skin biopsies. Fibroblasts ($8 \times 10^4/\text{well}$) were grown in 24 well plates with DMEM (Dulbecco's modified Eagle's medium) containing 10% fetal calf serum, 1% penicillin/streptomycin, and 1% L-glutamine as previously described [25]. Cell proliferation was followed by the MTT (3-[4,5-

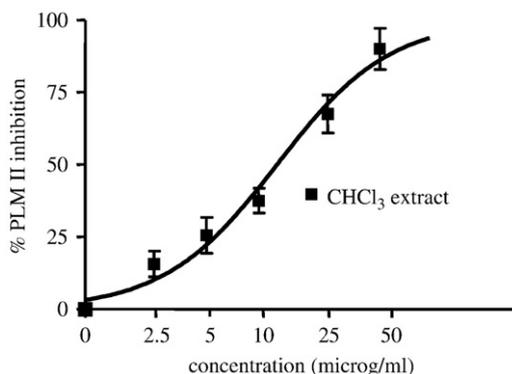


Fig. 2. Effect of the CHCl₃ fraction on PLM II. IC₅₀ value, expressed as the mean \pm SD of two experiments in triplicate, was 13.4 ± 1.7 $\mu\text{g/ml}$.

Table 1
Cytotoxicity vs anti-plasmodial activity of the *A. excelsa* stem bark methanolic extract and CHCl₃ fraction

Material	HF	D10	W2
	LC ₅₀ ±SD (µg/ml)	IC ₅₀ ±SD (µg/ml)	IC ₅₀ ±SD (µg/ml)
MeOH extract	116±19	4.6±1.4	2.8±0.88
CHCl ₃ fraction	46±7.5	3.1±0.15	2.1±0.58

dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) test [26], in the presence of the MeOH extract and CHCl₃ fraction (0–100 µg/ml) and LC₅₀ were calculated.

3. Results

The MeOH extract from the stem bark of *A. excelsa* inhibited parasite growth in a dose dependent manner, with IC₅₀ of 4.6 and 2.8 µg/ml on chloroquine-sensitive and -resistant strains, respectively (Fig. 1). After fractionation, the anti-plasmodial activity was retained by the CHCl₃ fraction (IC₅₀ of 3.1 and 2.1 µg/ml on D10 and W2, respectively). To investigate the mechanism of action, we evaluated whether the CHCl₃ fraction inhibited recombinant PLM II (Fig. 2), PLM IV, and the formation of β-haematin. CHCl₃ fraction reduced PLM II activity in a concentration dependent manner (IC₅₀ of 13.4±1.7 µg/ml, mean±SD), while was inactive against PLM IV. The CHCl₃ fraction inhibited also β-hematin formation dose dependently. At the highest dose tested (5 mg/ml) the inhibition was 37.7%.

As shown in Table 1, the MeOH extract and the CHCl₃ fraction were not devoid of cytotoxicity, but the MeOH extract was less cytotoxic than the CHCl₃ fraction (LC₅₀ of 116 µg/ml vs 45.5 µg/ml).

4. Discussion

In the present study it is reported that *A. excelsa* stem bark methanol extract is active *in vitro* against both chloroquine-sensitive and -resistant strains of *P. falciparum*, similar to *A. altissima* stem bark [10]. Our results suggest that extracts from *A. excelsa* stem bark exert anti-plasmodial activity through the inhibition of PLM II and by halting the haem detoxification process. Surprisingly, *A. excelsa* CHCl₃ extract was selective against PLM II, being inactive on PLM IV.

A. excelsa contains quassinoids [27], and β-carboline alkaloids, including canthin-6-one [28]. Canthin-6-one exhibited anti-plasmodial activity against W2 *Pf.* clone with IC₅₀ of 2.2 µg/ml, a value very close to that of the CHCl₃ fraction, as reported in the present work (Table 1). In a previous study, quassinoids were shown to exhibit anti-malarial effect through the inhibition of protein synthesis [9]. It cannot be excluded however that quassinoids and canthin-6-one and/or other β-carboline alkaloids act also against other targets such as the breakdown of haemoglobin and the haem detoxification to haemozoin. Either, other constituents of *A. excelsa* stem bark may act as the active principles.

Although the cytotoxicity studies revealed an anti-proliferative effect on mammalian cells by the MeOH extract, the LC₅₀ on human fibroblasts was 25 and 42 times higher than the IC₅₀ against *P. falciparum* D10 and W2 strains, respectively. Cytotoxicity increased when the crude MeOH extract was fractionated to give the CHCl₃ fraction, likely for the presence of higher concentrations of alkaloids which were found cytotoxic against tumor cell lines [29,30]. In conclusion *A. excelsa*, as other species of *Ailanthus*, possesses anti-plasmodial activity. The effect might be ascribable to the inhibition of haemoglobin cleavage and/or haem detoxification.

Acknowledgements

This work was funded by FIRST 2005 from the University of Milan.

References

- [1] Anis M, Sharma MP, Iqbal M. Pharmacol Biol 2000;38:241.
- [2] Shrimali M, Jain DC, Darokar MP, Sharma RP. Phytother Res 2001;15:165.
- [3] Ogura M, Cordell GA, Kinghorn AD, Farnsworth NR. Lloydia 1977;40:579.

- [4] Joshi BC, Pandey A, Chaurasia L, Pal M, Sharma RP, Khare A. *Fitoterapia* 2003;74:689.
- [5] Murakami C, Fukamiya N, Tamura S, Okano M, Bastow KF, Tokuda H, et al. *Bioorganic Med Chem* 2004;12:4963.
- [6] Rahman S, Fukamiya N, Ohno N, Tokuda H, Nishino H, Tagahara K, et al. *Chem Pharm Bull* 1997;45:675.
- [7] Kubota K, Fukamiya N, Tokuda H, Nishino H, Tagahara K, Lee KH, et al. *Cancer Lett* 1997;113:165.
- [8] Rahman S, Fukamiya N, Okano M, Tagahara K, Lee KH. *Chem Pharm Bull* 1997;45:1527.
- [9] Guo Z, Vangapandu S, Sindelar RW, Walker LA, Sindelar RD. *Curr Med Chem* 2005;12:173.
- [10] Bray DH, Boardman P, O'Neill MJ, Chan TH, Phillipson JD. *Phytother Res* 1987;1:22.
- [11] Okunade AL, Bikoff RE, Casper SJ, Oksman A, Goldberg DE, Lewis WH. *Phytother Res* 2003;17:675.
- [12] Wright CW. *J Ethnopharmacol* 2005;100:67.
- [13] Ersmark K, Samuelsson B, Hallberg A. *Med Res Rev* 2006;26:626.
- [14] Olliaro PL, Yuthavong Y. *Pharmacol Ther* 1999;81:91.
- [15] Egan TJ, Marques HM. *Coord Chem Rev* 1999;190–192:493.
- [16] Ohmoto T, Tanaka R, Nikaido T. *Chem Pharm Bull* 1976;24:1532.
- [17] Mitsunaga K, Koike K, Tanaka T, Ohkawa Y, Kobayashi Y, Sawaguchi T, et al. *Phytochemistry* 1994;35:799.
- [18] Trager W, Jensen JB. *Science* 1976;193:673.
- [19] Makler MT, Ries JM, Williams JA, Bancroft JE, Piper RC, Gibbins BL, et al. *Am J Trop Med Hyg* 1993;48:739.
- [20] Hill J, Tyas L, Phylip LH, Kay J, Dunn BM, Berry C. *FEBS Lett* 1994;352:155.
- [21] Dell'Agli M, Parapini S, Galli G, Vaiana N, Taramelli D, Sparatore A, et al. *J Med Chem* 2006;49:7440.
- [22] Bradford MM. *Anal Biochem* 1976;72:248.
- [23] Carroll CD, Patel H, Johnson TO, Guo T, Orlowski M, He ZM, et al. *Bioorg Med Chem Lett* 1998;8:2315.
- [24] Parapini S, Basilico N, Pasini E, Egan TJ, Olliaro P, Taramelli D. *Exp Parasitol* 2000;96:249.
- [25] Verotta L, Dell'Agli M, Giolito A, Guerrini M, Cabalion P, Bosisio E. *J Nat Prod* 2001;64:603.
- [26] Denizot F, Lang R. *J Immunol Methods* 1986;89:271.
- [27] Joshi BC, Pandey A, Sharma RP, Khare A. *Phytochemistry* 2003;62:579.
- [28] Cordell GA, Ogura M, Farnsworth NR. *Lloydia* 1978;41:166.
- [29] Kuo PC, Shi LS, Damu AG, Su CR, Huang CH, Ke CH. *J Nat Prod* 2003;66:1324.
- [30] De Feo V, Martino LD, Santoro A, Leone A, Pizza C, Franceschelli S, et al. *Phytother Res* 2005;19:226.