The antiplasmodium effects of a traditional South American remedy: *Zanthoxylum chiloperone var. angustifolium* against chloroquine resistant and chloroquine sensitive strains of *Plasmodium falciparum*

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Abstract: *Zanthoxylum chiloperone var. angustifolium* Engl., Rutaceae, is used in traditional medicine to treat fungal and protozoal infections in the central area of South America. Considering the increasing resistance of *Plasmodium falciparum* in malarial ridden areas, we explored the anti-plasmodial effects of three compounds isolated from *Z. chiloperone*. The pyranocoumarin trans-avicennol and the canthinone alkaloids, canthin-6-one and 5-methoxycanthin-6-one, were found to have IC50 on chloroquine/mefloquine resistant and sensitive strains of *P. falciparum* of 0.5-2.7, 2.0-5.3 and 5.1-10.4 μg/mL, respectively. Moreover, the formation of heme adducts by these compounds is described by a novel alternative method based on MS-CID methods. The alkylamide sanshool was also identified, for first time in this plant, in the dichloromethanic and ethanolic extracts and the extracts were found to be notably non-toxic and displayed good anti-plasmodial effects.

Keywords: alkylamides avicennol canthinone alkaloids Heme-drug adducts *Plasmodium falciparum* *Zanthoxylum chiloperone*

Introduction

In the majority of countries that cope with high malaria infection, medicinal plants contribute significantly to primary health care due to cultural traditions, and lack of access and affordability of pharmaceuticals. In addition, natural products are potential sources of new and selective agents for the treatment of important tropical diseases caused by protozoans (Karioti et al., 2009; Froelich et al., 2007; Luize et al., 2005). For example, *Zanthoxylum chiloperone var. angustifolium* Engl. (syn. *Fagara chiloperone* Engl. Ex Chod. & Hassl.), Rutaceae, is a dioic tree indigenous to the central and southern continent of South America, which is called “tembetary hu” and “mamicão” (Spichiger & Stutz de Ortega, 1987; Tabanez et al., 2005). A decoction of *Z. chiloperone* root and stem bark has been used in traditional medicine to treat malaria and for its emmenagogue and antirheumatic properties (Ferreira et al., 2007; Milliken, 1997). Recent studies have shown that the crude extract of the stem bark and leaves have

§Authors contributed equally to this work.
activity against *Trypanosoma cruzi* (Ferreira et al., 2011; Ferreira et al., 2002) and antifungal activity in vitro (Thouvenel et al., 2003). Further investigations demonstrate that canthinone type alkaloids, canthin-6-one and 5-methoxycanthin-6-one, are antifungal (Soriano-Agatón et al., 2005; Thouvenel et al., 2003) and effective in vivo against *Leishmania amazonensis* and *Trypanosoma cruzi* (Ferreira et al., 2011; Ferreira et al., 2007; Ferreira et al., 2002). Canthin-6-one has been suggested to be an inexpensive and safe treatment for use in long-term oral treatment as well as a good candidate against drug resistant strains of *T. cruzi*.

Other compounds were isolated from species of *Zanthoxylum* including the pyranocoumarin avicennol and alkylamides, such as the sanshools (Jang et al., 2008; Soriano-Agatón et al., 2005; Xiong et al., 1997; Yang, 2008). To date, there is a paucity of research on the biological activity of avicennol, which has been previously identified in *Z. elephantiasis* Macfad (Gray et al., 1977). Recent work with avicennol reports an induction of UDP-glucuronosyltransferases (UGT), specifically UGT1A1, which detoxifies xenobiotics (Chlouchi et al., 2007).

The alkylamides are a class of compounds which are well known in Asia, North America and Europe due to the tingling sensation on the tongue after ingestion of Sichuan pepper and *Echinacea purpurea* (Spelman et al., 2010). While many *Zanthoxylum* spp. are known to contain alkylamides, to the best of our knowledge there are no reports of the occurrence or identity of alkylamides in *Z. chiloperone*. Notably, these compounds have also been shown to be antiparasitic (Lozano et al., 1984), as well as insecticidal (Jacobson, 1948). Recent reports have shown in vitro and in vivo anti-plasmodial activity of alkylamides identical to, or similar to, the alkylamides occurring in other *Zanthoxylum* spp. (Spelman et al., 2010).

One of the mechanisms of action of antimalarial drugs is based on their interference with the heme detoxication pathway (Weissbuch & Leiserowitz, 2008). As a toxic, non-peptidic by-product of hemoglobin digestion, free heme must be sequestrated in the digestive vacuole. The biomineralization process involves the heme dimerization and the subsequent aggregation of dimers into hemozoin, an insoluble pigment. Antimalarial drugs are known to form adducts with heme, thus impairing heme polymerization and leading to the death of parasites at the trophozoite stage. Artemisinin for instance has been shown to form a covalent complex between heme and the alkylating species generated by the endoperoxide ring opening through Fe(II)-mediated catalysis (Robert et al., 2002). The free heme in its commercial form (hemin or hematin) is known to be suited for testing heme polymerization inhibition (Tekwani & Walker, 2005). Most of the protocols are based on spectrophotometry or radioactivity monitoring. (Garavito et al., 2007; Kurosawa et al., 2000; Rush et al., 2009). A novel method based on mass spectrometry can be used as an alternative method to detect heme-drug adducts (Figadère et al. 2010). It has been used to detect adducts between Fe(III)-heme and quinine, artemisinin and its derivatives. (Bilia et al., 2002; Pashynska et al., 2004). Our laboratory used mass spectrometry and in-source collision-induced dissociation (CID) to assess the stability of the adducts. In our method, heme is incubated with the compound and analyzed by mass spectrometry using an increasing fragmentor voltage to dissociate the formed adduct. A relative assessment of the adduct stability can be obtained and compared to one reference compound, like quinine.

Thus far there has been limited exploration of *Zanthoxylum chiloperone*’s chemistry and biological activity. The experiments continued herein describe the isolation of two alkaloids and a pyranocoumarin, the identification of an alkylamide and the anti-malarial activity of the crude extracts and the isolated compounds. In addition, using mass spectrometry and in-source CID to study the formation and stability of the heme adducts with avicennol and the canthinone alkaloids, we propose a possible basis for the antiplasmodial activity of these *Zanthoxylum* compounds.

**Material and Methods**

**General experimental procedures**

Yields refer to chromatographically and spectroscopically homogeneous materials, unless otherwise stated. Extraction was monitored by TLC and its derivatives. (Bilia et al., 2002; Pashynska et al., 2004). A novel method based on mass spectrometry can be used as an alternative method to detect heme-drug adducts (Figadère et al. 2010). It has been used to detect adducts between Fe(III)-heme and quinine, artemisinin and its derivatives. (Bilia et al., 2002; Pashynska et al., 2004). Our laboratory used mass spectrometry and in-source collision-induced dissociation (CID) to assess the stability of the adducts. In our method, heme is incubated with the compound and analyzed by mass spectrometry using an increasing fragmentor voltage to dissociate the formed adduct. A relative assessment of the adduct stability can be obtained and compared to one reference compound, like quinine.

**Plant material**

Stem bark of *Zanthoxylum chiloperone* var. *angustifolium* Eng., Rutaceae, was collected by Maria Elena Ferreira, in Paraguay near Piribebuy, Department
The antiplasmodium effects of a traditional South American remedy: *Zanthoxylum chiloperone* var. *angustifolium* against chloroquine resistant and

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of Cordillera and identified by N. Soria (Department of Botany, National University of Asuncion, Paraguay). A voucher specimen (AF 917) has been deposited at the Herbarium of Chemical Sciences Faculty, San Lorenzo, Paraguay.

**Extraction and isolation**

Powdered dried stem bark of *Z. chiloperone* var. *angustifolium* (1.3 kg) was basified with ammonia, extracted in a Soxhlet apparatus with CH₂Cl₂ and MeOH separately and successively for three days to afford 23 and 30 g of crude extracts, respectively. The yields of the dichloromethane and the methanol extracts were approximately 1.7 and 2.3%, respectively, of the plant powder. Both extracts were evaluated for antiplasmodial activity and the CH₂Cl₂ extract showed greater antimalarial activity. Therefore, the CH₂Cl₂ extract was subjected to silica gel flash column chromatography, eluted with CH₂Cl₂/ethyl acetate (8:2), to obtain 42 fractions. Fractions 10-18 were combined providing 3.5 g of *trans*-avicennol (1) a yellow crystal (yield of 15.2 and 0.3% respectively from the dichloromethane extract and plant powder). Fractions 21-30 were combined obtaining 3 g of canthin-6-one (2), a yellow crystalline powder (yield of 13 and 0.2%, respectively). Fractions 33-39 were combined yielded 1.5 g of 5-methoxy-canthin-6-one (3), a brown crystalline powder (with a yield of 6.5 and 0.11%, respectively). Physical and spectral data were used to determine the chemical structure of the compounds and compared to reference samples and literature values (1H, 13C NMR, MS, HRMS, IR).

The powered stem-bark (5 g) was basified with ammonia, extracted by ethanol maceration over two days, changing the solvent three times, for a yield of 2.3%. The compositions of the ethanol and the dichloromethane extracts were studied by HPLC-UV-MS method.

**Analysis by HPLC-UV-MS and MS/MS methods**

HPLC-UV-MS qualitative study

A LC/MSD 1100 series + 1100 series (Agilent Technologies) spectrometer was utilized in the HPLC study of the extracts by using a 150 mm x 4.6 mm stainless-steel column Sunfire (Waters) C18. The eluents were (A) H₂O+TFA 0.1% and (B) MeOH+0.1% TFA. Separations were performed at room temperature by solvent gradient elution from 50% A/50% B to 20% A/80% B in 20 min then to 100% B in 12 min at a flow rate of 1 mL/min.

The UV measurement was made at 240 and 260 nm. The injection volume was 5 μL. Peak identification was performed by comparison of the retention time (t₁), UV absorption and mass study at the different wavelengths with the compounds *trans*-avicennol (1), canthin-6-one (2), 5-methoxy-canthin-6-one (3) as references (Figure 1).

**Figure 1.** HPLC-UV Chromatogram of reference compounds (λ=260 and 240 nm).

Figure 2 illustrates the HPLC-UV chromatogram of the dichloromethane and alcohol extracts of stem bark extract of the plant *Z. chiloperone*.
HPLC-UV quantitative study

The quantification analysis was performed by external standard method, using a calibration curve constructed utilizing solutions containing different concentrations of the three reference samples (1-3). The stock solutions were prepared in a mixture MeOH+DMSO and then diluted with the mobile phase in various proportions. The calibration curves of the different samples represent the concentration “C” of each reference molecule versus the area under the peak “S”; this graphic presents a linearity behaviour \( C = a + bS \) in the interval of concentrations from 0.01 to 0.3 mg/mL.

All the samples for HPLC analysis were operated directly without any previous purification. A modular system comprised of 600 E controller + pump and a 2996 PDA UV (Waters) detector was utilized in the HPLC-UV quantification experiment.

Reference compounds stock solutions of 10, 50, 100, 150, 200, 300 µg/mL were utilized. Each determination was carried out in triplicate. The calibration curve showed the linearity of the detector over the tested range (10-300 µg/mL). The \( R^2 \) of the calibration curve is linear and 0.999. The quantitative evaluation of the peaks previously identified in the crude extracts was made by integration of the peak area and comparison with the area of the corresponding reference peak (Table 1).

| Table 1. Concentrations of compounds in the ethanol and dichloromethane extracts. |
|---------------------------------|---|---|---|
| (mg/g extract)                  | 1 | 2 | 3 |
| Stem bark DCM                   | 63.2 | 64.7 | 29.4 |
| Stem bark EtOH                  | 85.6 | 85.6 | 19.1 |

MS/MS study

To elucidate the presence of alkylamides, LC-ESI-MS/MS analysis was conducted on the crude extracts of \( Z. \) chiloperone ramulus cortex using the previously established structures of alkylamides identified in related \( Zanthoxylum \) spp. and the results of a previously validated LC-ESI-MS/MS method for the identification of alkylamides (Spelman et al., 2009). An ion trap mass spectrometer with electrospray ionization source (LCQ Advantage, ThermoFisher, San Jose, CA) was employed.

Figure 3. Selected ion chromatogram and MS/MS Spectra of dodeca-2,6,8,10-tetraenoic acid isobutylamide (4). A. The selected ion chromatogram illustrates the \( M^+ \) ion at \( m/z \) 248. B. The MS/MS spectra of the 248 \( M^+ \) ion identifies the compound as the alkylamide sanshool (4), dodeca-2,6,8,10-tetraenoic acid isobutylamide although the stereochemistry cannot be identified. The structure shown is that of the protonated \( (M^+) \) form of sanshool 4.
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The solvent gradient, which was a minor variation on that previously published (Spelman et al., 2009), was as follows, where solvent A is aqueous acetic acid (17 mM, original pH 2.74) and solvent B is neat HPLC grade acetonitrile. For 

Hemolytic activity

In order to confirm that the antimalarial activities measured by [3H] hypoxanthine incorporation are due to the intrinsic antiparasite property of the experimental compounds and not due to hemolytic activity, a standard hemolysis assay was run. After 48 h incubation time in the presence of the various test compounds, the hemolysis of non-infected erythrocytes was measured at 530 nm
incubated for 48 h at concentrations up to 100 μg/mL.

Table 2. IC50 of Z. chiloperone isolated compounds and extracts.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>P. falciparum IC50 (μg/mL)</th>
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<tr>
<td></td>
<td>F32</td>
</tr>
<tr>
<td><strong>Trans-avicennol (1)</strong></td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Canthin-6-one (2)</strong></td>
<td>2.0</td>
</tr>
<tr>
<td><strong>5-methoxycanthin-6-one (3)</strong></td>
<td>10.4</td>
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Table 3. Cell survival of MCR5 cells incubated with Z. chiloperone isolated compounds and extracts.

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<tr>
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<td>4.4</td>
</tr>
<tr>
<td><strong>Canthin-6-one (2)</strong></td>
<td>9.4</td>
</tr>
<tr>
<td><strong>MeOH extract</strong></td>
<td>&gt;100</td>
</tr>
<tr>
<td><strong>DCM extract</strong></td>
<td>12.3</td>
</tr>
<tr>
<td><strong>EtOH extract</strong></td>
<td>13.0</td>
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</tbody>
</table>

Cytotoxic activity

The cytotoxicity of the plant extracts was determined using the colorimetric methylthiazoletetrazolium (MTT) assay (Mosmann, 1983). MRC5 cells were incubated for an extended period (five days) in the presence of trans-avicennol 1, canthin-6-one 2, 5-methoxycanthin-6-one 3, and the dichloromethane, ethanol and methanol extracts of Z. Chiloperone (Table 3), and scored as a percentage of absorbance reduction at 540 nm of treated cultures versus untreated control cultures. IC50 values on cell growth were obtained from the drug concentration-response curves and expressed as the means±the standard deviations determined from triplicate independent experiments.

Table 3. Cell survival of MCR5 cells incubated with Z. chiloperone isolated compounds and extracts.

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<td><strong>EtOH extract</strong></td>
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</tbody>
</table>

Results and Discussion

Qualitative and quantitative analysis of Z. chiloperone stem bark dichloromethane and ethanol extracts

Analysis using HPLC-UV-MS methods

The composition of each organ of Z. chiloperone was analyzed by HPLC-UV-MS study of the ethanol and dichloromethane extracts of stem bark. The qualitative studies identified the main compounds in Z. chiloperone, such as the coumarin trans-avicennol 1 and canthin-6-one alkaloids, i.e. canthin-6-one 2, 5-methoxy-canthin-6-one 3. The compounds were identified by their retention times (tR) and by their mass spectra respectively (see also Figures 1 and 2: chromatographic profiles of the reference compounds and profiles of the respective ethanol and dichloromethane extracts).

![Chemical structures](image)

The quantitative analysis was performed by the external standard method, using a calibration curve constructed utilizing solutions containing different concentrations of the three reference samples, trans-avicennol 1, canthin-6-one 2 and 5-methoxy-canthin-6-one 3. The concentrations of trans-avicennol 1 in the dichloromethane and ethanol extract of the stem bark are 63.2 and 85.6 mg/g extract respectively, while the concentrations of canthin-6-one 2 in the same extracts are 64.7 and 85.6 mg/g extract respectively, finally the concentrations of 5-methoxycanthin-6-one 3 are 29.4 and 19.1 mg/g of extract respectively (Table 1).

MS/MS of Z. chiloperone demonstrates the presence of alkylamides

Previous reports have shown a substantial profile of alkylamides occurring in Zanthoxylum spp. (Jang et al., 2008; Xiong et al., 1997; Yang, 2008). However, to our knowledge, there have been no reports on the presence of alkylamides in Z. chiloperone. To elucidate the presence of alkylamides, LC-ESI-MS/MS analysis was conducted on the crude extracts of Z. chiloperone ramulus cortex using a previously validated method (Spelman et al., 2009). Figure 3A illustrates a selected ion chromatogram obtained from analysis of the 1:2 dry stem bark of Z. chiloperone extract. The ion corresponds to the m/z of the protonated (M+1) forms of one of the alkylamides in Z. chiloperone of MW 247. Figure 3B illustrates the MS/MS spectrum obtained by (CID) of the M+ ion. For ease of reference, Figure 3B and Table 4 illustrate the primary fragments obtained. These major groups of fragments are consistent with those previously observed for alkylamides (Spelman et al., 2009). They confirm the presence of compound 4, dodeca-2,6,8,10-tetraenoic acid isobutylamide, also known as sanshool, in Z. chiloperone. Note that it is not possible to distinguish E/Z isomers with the LC-MS method employed here.

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Zanthoxylum chiloperone

The antiplasmodium effects of a traditional South American remedy: Fragments formed by Collisionally Induced Dissociation (MS-MS) of the M$^+$ ion of multiple active compounds and possible unknown modes of intriguing finding and suggest that there may be various are used to treat Chagas disease, this is an chiloperone and falciparum in vitro et al., 2010). Considering that traditional extracts of alkylamide(s), previously shown to be active against one type compounds, the pyranocoumarin, as well as the This is possibly due to the combination of the canthin-6-one 2-methylbutylamide; fragments are carbocations that correspond to the alkyl chain of the alkylamide and are formed by loss of the amide portion (isobutylamide or ii fragments correspond to the alkyl chain of the alkylamide and are formed by the loss of the amide portion of the molecule and saturation of one of the double bonds on the akyl chain. The m/z value of these fragments is +2 greater than the group ii fragments; The group iv fragments correspond to the protonated alkylamide minus the N-alky group; The group v fragments correspond to the protonated alkylamide minus various portions of the N-alkyl group (see Fig. 3 for the bonds at which fragmentation occurs); The group vi fragments correspond to the varying carbocation fragments formed after loss of various portions of the C-alkyl group.

In vitro biological activity of isolated compounds and extracts of Z. chiloperone towards P. falciparum

After isolation of trans-avicennol 1, canthin-6-one 2 and 5-methoxycanthin-6-one 3, the half maximal inhibitory concentrations (IC50) of extracts and the previously listed compounds were assessed by determining the concentration inhibiting 50% of parasite growth. The IC50 were determined against the Colombian chloroquine-resistant FcB1 strain, the Brazilian mildly chloroquine-resistant strain PFB, the multi-drug and chloroquine-resistant strain K1 from Thailand, and the chloroquine-sensitive, but mefloquine resistant Tanzanian strain F32. Figure 5 illustrates the IC50 for avicennol 1, canthin-6-one 2 and 5-methoxycanthin-6-one 3 on the Plasmodium falciparum strain F32. These data show that F32 is the most sensitive strain to avicennol 1 and canthin-6-one 2 (0.5 and 2.0 μg/mL, respectively). Further testing of additional P. falciparum strains demonstrated that these compounds all had approximately the same activity on K1, PFB and FcB1 strains. Table 2 shows the results on parasite growth in tabulated form. Note that the crude extracts of Z. chiloperone demonstrated robust activity. This is possibly due to the combination of the canthin-6-one type compounds, the pyranocoumarin, as well as the alkylamide(s), previously shown to be active against P. falciparum in vitro and P. yoelii yoelii in vivo (Spelman et al., 2010). Considering that traditional extracts of Z. chiloperone are used to treat Chagas disease, this is an intriguing finding and suggest that there may be various modes of Plasmodium inhibition due to the presence of multiple active compounds and possible unknown compounds as well.

Figure 5. IC50 of isolated compounds from Z. chiloperone against P. falciparum F32. Avicennol shows an IC50 of 0.5 μg/mL; canthin-6-one and 5-methoxycanthin-6-one show IC50 of 2.0 and 10.4, respectively.

IC50 values for the positive control chloroquine are also listed in Table 2. Note that the IC50 for our K1 clone of P. falciparum were considerably less than those published by Elueze et al. (1996) and Fivelman et al. (2007), but higher than Sharrock et al. (2008).

Study of hemolytic activity of compounds isolated from, and extracts of Z. chiloperone

In order to confirm that the antimalarial activities measured by [3H]-hypoxanthine incorporation are due to the intrinsic antiparasite property of the experimental compounds and not due to hemolytic activity, a standard hemolysis assay was run. After 48 h incubation time in the presence of the various test compounds, the hemolysis of non-infected erythrocytes was measured at 530 nm. Results demonstrated that trans-avicennol 1, canthin-6-one 2 and 5-methoxycanthin-6-one 3, as well as the dichloromethane, ethanol and methanol extracts of Z. chiloperone incubated for 48 h at concentrations up to 100 μg/mL, had no haemolytic effect on erythrocytes.

![Diagram](Image)

Table 4. Fragments formed by Collisionally Induced Dissociation (MS-MS) of the M$^+$ ion of various Z. chiloperone alkylamides.

<table>
<thead>
<tr>
<th>Designation, name, m/z</th>
<th>group i$^+$</th>
<th>group ii$^+$</th>
<th>group iii$^+$</th>
<th>group iv$^+$</th>
<th>group v$^+$</th>
<th>group vi$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>248$^+$</td>
<td>175</td>
<td>147</td>
<td>149</td>
<td>206</td>
<td>220, 234</td>
<td>81, 93, 121, 133</td>
</tr>
</tbody>
</table>

1The number beneath the letter designation indicates the m/z value for the M$^+$ ion; 2Proposed structure for compound E based on retention time and MS/MS fragmentation; 3RI corresponds to relative intensity; 4The group ii fragments correspond to acylium ions as shown in Figure 3; 5The group ii fragments correspond to the alkyl chain of the alkylamide and are formed by loss of the amide portion (isobutylamide or 2-methylbutylamide); 6The group iii fragments correspond to the alkyl chain of the alkylamide and are formed by the loss of the amide portion of the molecule and saturation of one of the double bonds on the akyl chain. The m/z value of these fragments is +2 greater than the group ii fragments; 7The group iv fragments correspond to the protonated alkylamide minus the N-alky group; 8The group v fragments correspond to the protonated alkylamide minus various portions of the N-alkyl group (see Fig. 3 for the bonds at which fragmentation occurs); 9The group vi fragments correspond to the varying carbocation fragments formed after loss of various portions of the C-alkyl group.

![Image](Image)

Cytotoxicity assay

To probe for cytotoxicity, MRC5 cells were incubated for an extended period (five days) in the presence of trans-avicennol 1, canthin-6-one 2 and 5-methoxycanthin-6-one 3, and the dichloromethane, ethanol and methanol extracts of Z. chiloperone. Table 3 shows the results of these assays. Trans-avicennol 1 had the most cytotoxic effect on MRC5 cells at an IC50 of 4.4 μg/mL. The canthinone alkaloids, canthin-6-one 2 and 5-methoxycanthin-6-one 3, were observed to have IC50 at 12.1 and 12.3 μg/mL, respectively. Supporting the frequent use of this plant species in traditional medicine, the crude extracts demonstrated less cytoxicity with the DCM extract and the ethanol extract showing 12.5 and 13.0 μg/mL IC50, respectively. The hydrophilic methanol extract had no detectable effect on cell survival up to 100 μg/mL.

MS-CID detection of the heme-compound (1, 2, 3) adducts

The formation of adducts between compounds and heme is an indication of the ability of the compound to interfere with hematin formation process and thus to show a potential antimalarial activity. Mass spectra from the incubates with heme (Figure 4) show a clear adduct for each of the compounds. The stability of the adducts vs fragmentor voltage.

Conclusion

The studies herein report the quantization, identification and isolation of compounds new to Zanthoxylum chiloperone including the pyranocoumarin avicennol 1 and the canthinone alkaloids canthin-6-one 2 and 5-methoxycanthin-6-one 3. In addition, we identified sanshool, dodeca-2,6,8,10-tetraenoic acid isobutyramide 4, in this species. We report a good antiplasmodial effect on chloroquine resistant and sensitive strains of P. falciparum for the pyranocoumarin avicennol 1 (LD50 of 0.5-2.7 μg/mL) and the canthinone alkaloids (LD50 of 2.0-5.3 μg/mL and 5.1-10.4 μg/mL) for canthin-6-one 2 and 5-methoxycanthin-6-one 3, respectively. The generation of adducts, heme-avicennol and heme-canthinones, suggest the basis that this activity may be at least partly explained through the heme detoxification pathway. Intriguingly, the dichloromethane and ethanol extracts showed robust effects on the chloroquine-resistant strain K1, and the chloroquine-sensitive, but mefloquine resistant Tanzanian strain F32 of P. falciparum. Considering these data, future improvements in antimalarial chemotherapy based on avicennol and the canthinone alkaloids could generate analogs with increased selectivity and lowered resistance index, and opens new horizons for targeting the increased mortality due to drug-resistant malaria.

Acknowledgements

The authors thank the European Community for the financial support (Marie Curie Early Stage Training Fellowship of the European Community’s Sixth Framework Programme: contract BioMedChem (for G. CT., K. S. and K. MD.). Thanks are due to Mrs. A. Solgadi (Samm) for MS experiments and Dra Nashira Campos Vieira (University of Brasilia) for the portuguese traduction.

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