



Short communication

## Antiplasmodial evaluation of *Anacardium occidentale* and alkyl-phenols

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### ABSTRACT

The ethanol crude extract from cashew (*Anacardium occidentale* L. Anacardiaceae) displayed significant antiplasmodial activity (IC<sub>50</sub> 0.577 µg/ml). Liquid chromatography-high resolution Mass spectrometry analysis was performed to identify the main compounds existing in the ethanol extract. The occurrence of anacardic acids, cardols, and 2-methylcardols derivatives was confirmed in the extract. The IC<sub>50</sub> obtained, when the main isolated compounds were evaluated in *Plasmodium falciparum* D6 strain, ranged from 5.39 µM to >100 µM. Tested here for the first time, the data showed that cardol triene 1 (IC<sub>50</sub> = 5.69 µM) and 2-methylcardol triene 4 (IC<sub>50</sub> = 5.39 µM) demonstrated good antimalarial activity. In conclusion, *Anacardium occidentale* nuts presented relevant biological potential, and further studies should be considered.

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### Introduction

Anacardiaceae is a plant family of mostly tropical trees, comprising around seventy genera and 600 species found predominantly in tropical areas of Africa, Asia, and America (Mitchell and Mori, 1987; Wannan, 2006). Phytochemically, the family Anacardiaceae is known by biosynthesis of flavonoids, triterpenes, steroids, xanthenes, and alkyl-phenols (Correia et al., 2006).

*Anacardium occidentale* L. (the “cashew”) is recognized as an indispensable source of alkyl-phenols, which were isolated from its fruit (the “cashew nuts”). These compounds have several biological activities described among them: antioxidant, larvicidal, cytotoxicity to cancer cell lines, antibacterial, molluscicidal, and schistosomicidal (Kubo et al., 1986; Himejima and Kubo, 1991; Kubo et al., 2011; Oliveira et al., 2011; Alvarenga et al., 2016). They also showed inhibitory activities in enzymatic assays, when evaluated against tyrosinase, acetylcholinesterase, α-glucosidase, aldose reductase, invertase, 15-lipoxygenase, and xanthine oxidase (Toyomizu et al., 1993; Kubo et al., 1994; Shobha et al., 1994; Oliveira et al., 2011; Masuoka et al., 2015).

As reported by the World Health Organization, malaria affected 216 million people in 2016. The number of estimated deaths, in 2016, was 445 thousand, and the records point to 407 thousand deaths in Africa alone (WHO, 2017). Additionally, drug resistance, particularly to artemisinin-based combination therapies is also an increasing concern (Haldar et al., 2018). These facts make the continued search for new antimalarials important.

Because of the promising activity presented by the alkyl-phenols when evaluated against the adult worms of *Schistosoma mansoni*, but also because cashew seed oil is used in folk medicine to treat malaria (Duke, 2000), we sought to assess the extract and alkyl-phenols from *A. occidentale* against *Plasmodium falciparum*, the causative agent of malaria.

### Materials and methods

We purchased cashew nut and apple (*Anacardium occidentale* L., Anacardiaceae) from “Varejão Irmãos Patrocínio” market, Franca, São Paulo State, in October 2013. Prof. Dr. Milton Groppo authenticated the plant sample, and a voucher specimen (SPFR 16040) has been stored in the Herbarium of the Department of Biology, Faculdade de Ciências e Letras de Ribeirão Preto, University of São Paulo, Brazil (Herbarium SPFR).

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The air-dried and powdered cashews (34.9 g) were extracted with ethanol using ultrasound, yielding 12 g of crude extract. Previously, the ethanol extract (5 g) was purified, as described by Alvarenga et al. (2016), resulting in seven compounds: **1** (63.9 mg, cardol triene), **2** (45.8 mg, cardol diene), **3** (8.5 mg, cardol monoene), **4** (29.9 mg, 2-methylcardol triene), **5** (11.0 mg, 2-methylcardol diene), **6** (8.9 mg, anacardic acid triene), and **7** (4.1 mg, anacardic acid diene).

The crude extract was analyzed by high-performance liquid chromatography-high resolution Mass spectrometry (HPLC-HRMS) using a micrOTOF-Q II-ESI-TOF Mass Spectrometer (Bruker Daltonics), Shimadzu HPLC system with an LC 20 AD pump, automatic injection SIL20AHT, DAD (200–600 nm) detector, and using a C18 column (Phenomenex® Luna, 5  $\mu$ m 250  $\times$  4.6 mm). The following conditions were applied: capillary voltage 3.5 kV, dry temperature 220 °C, nebulizer gas 60 psi, dry gas 10 l/min, mass range 50–1300 Da Nitrogen was used as drying, nebulizing and collision gas. A linear solvent gradient from 30% H<sub>2</sub>O/0.1% acetic acid (solvent A) and 70% methanol (solvent B) to 100% solvent B over 10 min followed by 5 min 100% solvent B, with 1.0 ml/min flow rate. All analyses were processed using Bruker Daltonics Data Analysis 4.3 software.

The *Plasmodium falciparum* D6 strains (chloroquine-sensitive), were maintained, with slight adaptations, according to Trager and Jensen (1976). They were cultivated in culture flasks at 37 °C in an atmosphere of 5% O<sub>2</sub>, 5% CO<sub>2</sub>, and 90% N<sub>2</sub>, with group A-positive human erythrocytes at 2% hematocrit in RPMI 1640 medium, supplemented with Albumax II (0.5%), glucose (3 g/l), hypoxanthine (45  $\mu$ g/l), and gentamicin (50  $\mu$ g/l). Every 3–4 days, the percent of parasitemia was calculated by light microscopy counting of 500 erythrocytes on a Giemsa-stained blood smear. Then, infected erythrocytes were transferred to fresh medium containing erythrocytes.

Stock solutions of the compounds (**1–7**), and crude extract were prepared at a concentration of 10 mM and 180  $\mu$ g/ml, respective, and were dissolved in 1 ml DMSO. The drugs atovaquone (ATV), ELQ-300, and chloroquine (CQ) were used as positive controls and were prepared in a 10 mM stock solution. They were dissolved in 1 ml DMSO, exception chloroquine, which was prepared in 1 ml water. Serial dilutions of test compounds, extract, and positive controls were made with RPMI 1640 medium in 96 well plates and combined with stock parasite culture and erythrocytes to yield a final 2% hematocrit and 0.2% parasitemia. The maximum percentage of DMSO, after the serial dilution, was 0.1%. Negative controls with DMSO-only were included on each assay plate. After a 72 h growth period, the assay was performed as previously described (Smilkstein et al., 2004).

Antiplasmodial activity was obtained using IC<sub>50</sub> value (50% *P. falciparum* growth inhibition), which was calculated with Graph-Pad Prism software by plotting the % of observed growing against the logarithm of the sample concentrations and curve fitting by nonlinear regression.

## Results and discussion

The obtained data (Table 1) indicated that the cashew crude ethanol extract showed good antiplasmodial activity, when compared to Lemma et al. (2017) ranked data, which established that good activity is observed when the IC<sub>50</sub> falls in the range of 0.1–1  $\mu$ g/ml.

The bioactive extract was analyzed by LC-HRMS, aiming better characterization of the crude extract components, since, in most cases, the traditional phytochemical study results only in the isolation of the abundant compounds, thus providing additional

**Table 1**  
Antiplasmodial activity of crude ethanol extract of cashew and alkyl-phenols (**1–7**).

Sample	<i>Plasmodium falciparum</i> D6 strain <sup>a</sup>		95% CI <sup>b</sup>
	IC <sub>50</sub> [ $\mu$ g/ml]	IC <sub>50</sub> [ $\mu$ M]	
Crude ethanol extract	0.577	–	0.47–0.71
<b>1</b>	1.79	5.69	4.35–7.40
<b>2</b>	8.79	27.83	21.79–40.54
<b>3</b>	>31.8	>100	na <sup>c</sup>
<b>4</b>	1.77	5.39	4.61–6.28
<b>5</b>	13.80	41.82	31.83–69.16
<b>6</b>	22.19	64.89	42.84–142
<b>7</b>	7.32	21.28	14.59–36.96
CQ	0.0077	0.015	0.013–0.016
ATV	0.00007	0.0002	0.00016–0.00022
ELQ-300	0.0016	0.0033	0.0029–0.0038

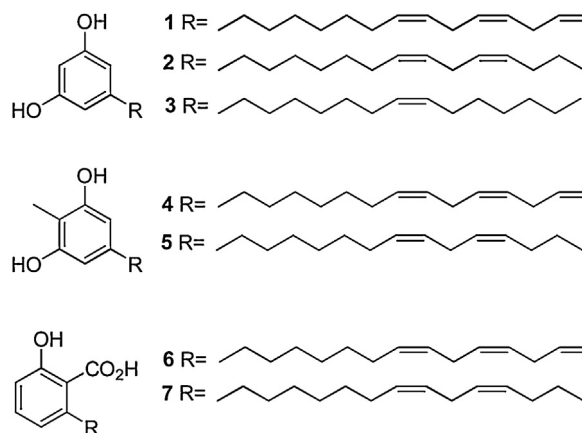
<sup>a</sup> Results are from three separate experiments, each run in two-four replicates.

<sup>b</sup> Confidence interval.

<sup>c</sup> Not applicable.

information about the metabolite profile. The LC-HRMS data of crude extract were analyzed, and the peaks with high intensity in the HRMS chromatogram were selected for detailed examination. The mass spectra were investigated to identify the ions corresponding to each peak. In all cases, one or two protonated and/or sodiated ions ([M+H]<sup>+</sup> and/or [M+Na]<sup>+</sup>) were observed. The exact mass obtained was used to determine the possible molecular formulae, and these formulae were searched in Natural Products Dictionary online database together with *Anacardium* and confirmed the presence of the seven alkyl-phenols, which were usually found in cashew nuts (Table 2).

The results of the *in vitro* tests with isolated compounds against *P. falciparum* D6 strain were also presented in Table 1. The IC<sub>50</sub> of compounds ranged from 5.39  $\mu$ M to >100  $\mu$ M. According to Batista et al. (2009), the obtained alkyl-phenols showed good activity as for **1** (IC<sub>50</sub> = 5.69  $\mu$ M) and **4** (IC<sub>50</sub> = 5.39  $\mu$ M), and moderate activity as for compounds **7** (IC<sub>50</sub> = 21.28  $\mu$ M), **2** (IC<sub>50</sub> = 27.83  $\mu$ M), **5** (IC<sub>50</sub> = 41.82  $\mu$ M), and **6** (IC<sub>50</sub> = 64.89  $\mu$ M).



The presence of a carboxyl group in phenol ring was associated with a decrease in the antiplasmodial activity in the case of anacardic acid triene (**6**), when compared to cardol triene (**1**) and 2-methylcardol triene (**4**). Additionally, the existence of a double bond between C-14 and C-15, and C-11 and C-12 are essential for the *in vitro* activity of compounds **1** and **4**. In general, a decrease in the number of double bonds in the side chain decreased the activity as in compounds **1**, **2** and **3**, besides **4** and **5**, although this was not observed for compounds **6** and **7**, which showed the opposite pattern. Compounds **6** and **7** have a carboxyl in the aromatic ring, which may explain in part these finds.

**Table 2**  
LC-HRMS analysis of cashew ethanol crude extract.

Accurate <i>m/z</i>	<i>t<sub>R</sub></i> (min)	Quasi-form	Suggested formulae	Tentative identification	Mass error ppm
315.2319	4.8	[M+H] <sup>+</sup>	C <sub>21</sub> H <sub>31</sub> O <sub>2</sub>	Cardol triene	−1.59
317.2468	4.8	[M+H] <sup>+</sup>	C <sub>21</sub> H <sub>33</sub> O <sub>2</sub>	Cardol diene	−4.10
329.2471	4.8	[M+H] <sup>+</sup>	C <sub>22</sub> H <sub>33</sub> O <sub>2</sub>	2-methylcardol triene	−3.04
335.2959	5.1	[M+H] <sup>+</sup>	C <sub>22</sub> H <sub>39</sub> O <sub>2</sub>	2-methylcardol	−2.68
343.2262365.2081	5.1	[M+H] <sup>+</sup> [M+Na] <sup>+</sup>	C <sub>22</sub> H <sub>31</sub> O <sub>3</sub>	Anacardic acid triene	3.20–3.29
347.2579369.2398	5.5	[M+H] <sup>+</sup> [M+Na] <sup>+</sup>	C <sub>22</sub> H <sub>35</sub> O <sub>3</sub>	Anacardic acid monoene	−2.022.17
371.2567	5.5	[M+Na] <sup>+</sup>	C <sub>22</sub> H <sub>37</sub> O <sub>3</sub>	Anacardic acid	−1.35

The antimalarial activity of the cardol and 2-methylcardol derivatives has not previously been reported, and the inhibitory potencies of compounds **1** and **4** are notable.

Anacardic acid derivatives isolated from *Viola websteri*, 6-(8'Z-pentadecenyl)-salicylic acid and 6-(8'Z, 11'Z, 14'Z-heptadecatrienyl)-salicylic acid, for which antimalarial activity has been previously described with IC<sub>50</sub> values of 10.1 and 13.3 μM (Lee et al., 2009). Compound 6-(8'Z-pentadecenyl)-salicylic acid was also evaluated *in vivo* on *Plasmodium berghei* infections, and the study indicated moderate antimalarial activity (Chung et al., 2009).

Cui and coworkers (2008) proposed that anacardic acid affect *P. falciparum* due to partial inhibition of recombinant PfGCN5 histone acetyltransferase (HAT), causing problems to the parasite transcription. Also, the anacardic acid exhibited an IC<sub>50</sub> of around 30 μM in parasite strains.

In conclusion, our data add further evidence that cashew is a rich source of bioactive compounds. The crude ethanol extract showed good antiplasmodial activity, thus presenting relevant potential. The activity profile of the cardol and 2-methylcardol derivatives tested here against *P. falciparum* for the first time, suggested potential and provides early structure activity relationship information. Further studies based on chemical modifications in the alkyl-phenols could be examined to further improve antiplasmodial activity.

**Authors' contributions**

VMMG and TAA contributed by running the laboratory work. MG identified the plant material. AHJ, WRC, and MLAS collaborated with the phytochemical investigation. MJS cooperated with the biological studies. PMP wrote the paper with contributions from AHJ, WRC, and MLAS. PMP and MKR designed the study and critically read the manuscript. All the authors have read the final manuscript and approved the submission.

**Ethical disclosures**

**Protection of human and animal subjects.** The authors declare that no experiments were performed on humans or animals for this study.

**Confidentiality of data.** The authors declare that no patient data appear in this article.

**Right to privacy and informed consent.** The authors declare that no patient data appear in this article.

**Conflicts of interest**

The authors declare no conflicts of interest.

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