In vitro and in vivo antimalarial activity and cytotoxicity of extracts, fractions and a substance isolated from the Amazonian plant *Tachia grandiflora* (Gentianaceae)

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Tachia *sp. are used as antimalarials in the Amazon Region and in vivo antimalarial activity of a* Tachia *sp. has been previously reported.* Tachia grandiflora *Maguire and Weaver is an Amazonian antimalarial plant and herein its cytotoxicity and antimalarial activity were investigated. Spectral analysis of the tetraoxygenated xanthone decussatin and the iridoid aglyone amplexine isolated, respectively, from the chloroform fractions of root methanol and leaf ethanol extracts was performed. In vitro inhibition of the growth of* Plasmodium falciparum *Welch was evaluated using optical microscopy on blood smears. Crude extracts of leaves and roots were inactive in vitro. However, chloroform fractions of the root and leaf extracts [half-maximal inhibitory concentration (IC_{50}) = 10.5 and 35.8 µg/mL, respectively] and amplexine (IC_{50} = 7.1 µg/mL) were active in vitro. Extracts and fractions were not toxic to type MRC-5 human fibroblasts (IC_{50} > 50 µg/mL). Water extracts of the roots of* T. grandiflora *administered by mouth were the most active extracts in the Peters 4-day suppression test in* Plasmodium berghei-*infected mice. At 500 mg/kg/day, these extracts exhibited 45-59% inhibition five to seven days after infection.* T. grandiflora *infusions, fractions and isolated substance have potential as antimalarials.*

Key words: amplexine (djalonenol) - decussatin - *Plasmodium falciparum* - *Plasmodium berghei* - human fibroblasts

Geographic distribution of Tachia spp - *Tachia* Aubl. (Gentianaceae) was first described by the botanist Aublet in 1775 based on plants collected in French Guyana. Before 1975, only the type species *Tachia guianensis* Aubl. was known. Recent studies show that *T. guianensis* occurs only in French Guyana and Suriname (Peters et al. 2005). Today, it is accepted that *Tachia* is comprised of 13 closely related understory shrubs or small tree species (Peters et al. 2005), which occur also in the wet forests of the Amazon Basin and the lower elevations of mountainous areas of Peru, Ecuador, Colombia, Venezuela and Brazil (7 species) and Panama (1 species) (Peters et al. 2005). *Tachia grandiflora* (Maguire & Weaver 1975), studied herein, is widespread in wet tropical forests of the central and eastern part of the Amazon Basin and northwestern South America and from French Guyana and Suriname in the north to central parts of the state of Amazonas (AM) in Brazil to the west and south [see map in Peters et al. (2005)].

Traditional use of Tachia spp as antimalarials - Ethnobotanical and ethnopharmacological publications have described the traditional uses of *Tachia* spp as antimalarials and febrifuges in The Guyanas, Brazil, Colombia and Peru (Milliken 1997). However, in many Brazilian (Carvalho & Krettli 1991, Brandão et al. 1992, Milliken 1997, Mors et al. 2000, Krettli et al. 2001), Colombian (Schultes & Raffauf 1990) and Peruvian (Milliken 1997) studies the plants collected are incorrectly identified as the type species of the genus, *T. guianensis* Aubl.

T. grandiflora - We became interested in studying the local *caferana* plant, *T. grandiflora,* based on earlier reports by the Dr Antoniana Krettli group (Oswaldo Cruz Foundation, state of Minas Gerais, Brazil) in which the water extract of roots of a *Tachia* sp. exhibited significant in vivo activity in a mouse model of malaria. *Tachia* spp are rare, sparsely populated plants in the Amazon forests. We initially conducted studies on the propagation of this plant from stem cuttings (Silva et al. 2006). Pio Corrêa (1926) reported that *caferana* extracts were toxic. Polar

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extracts of *T. grandiflora* were not toxic to *Artemia franciscana* in the brine shrimp assay (Quignard et al. 2003). In another study, extracts of *T. grandiflora* at 500 µg/mL exhibited moderate toxicity (7-64% lethality) to larvae of *Aedes aegypti* (Pohlit et al. 2004). Also, extracts of *T. grandiflora* were highly active inhibitors of the growth of cancer tumour cell lines (Pohlit et al. 2007).

Antimalarial plants such as *T. grandiflora* are potential sources of drug leads against *Plasmodium* spp (Andrade-Neto et al. 2007, Schmidt et al. 2012a, b). Recently, we isolated the tetra-oxygenated xanthone decussatin (1) and a rare seco-iridoid monoterpene aglycone, djalonenol (amplexine) (2), from *T. grandiflora* (Pohlit et al. 2012). In the present work, the in vitro and in vivo antiplasmodial activity and cytoxicity of the extracts, fractions and chemical components of the leaves and roots of the central Amazonian *caferana* plant, *T. grandiflora*, were investigated. Spectroscopic characterisation of the isolates 1 and 2 is also presented.

MATERIALS AND METHODS

Chemicals - All solvents used for extraction, partitioning and chromatography were fractionally distilled prior to use. Solvents for NMR were purchased from Sigma-Aldrich (St. Louis, USA).

Instrumentation - Medium pressure liquid chromatography (MPLC) was performed using a Büchi System with Pump model 688, Gradient Former model 687, ultraviolet visible spectroscopy and fraction collector model 684 and a normal phase column with 40-63 µm particle size. ^{1}H-NMR, ^{13}C-NMR, DEPT 135, ^{1}H-^{1}H COSY and HMQC spectra were acquired on a Bruker DPX 300 (300 MHz) in $CDCl_3$/TMS or $(CD_3)_2CO$/TMS. FT-IR spectra were acquired on a Bomem model M 102 spectrometer. Electronic ionization-gas chromatography-mass spectrometry (EI-GC-MS) was performed on a Hewlett-Packard HP 5890 series gas chromatograph coupled to mass detector HP 5971 operating at an ionization energy of 70 eV.

Plant material - Plant materials were collected in September and October, 2000 in National Institute for Amazonian Research's (INPA) Campina and Adolpho Ducke Forest Reserves which are located in greater Manaus, AM. Voucher specimens were deposited at the INPA Herbarium under the accessions 208104 (collector AM Pohlit) and 205948 (collector AM Pohlit). Identification of the plant samples as *T. grandiflora* Maguire and Weaver (Gentianaceae) was corroborated by LS (co-author of the present paper). Roots and mature leaves were separately dried in the shade and ground to fine powders.

Extraction and isolation of 1 - Dried, powdered roots were continuously extracted in a Soxhlet apparatus with methanol (3 × 6 h). The extracts were combined and evaporated under vacuum using a rotary evaporator with heat bath (≤ 40ºC) and then freeze-dried. The resulting dry methanol extract (5 g) was dissolved in methanol-distilled water (9:1) and partitioned with hexanes, then chloroform. After total evaporation, the chloroform fraction was analysed by thin-layer chromatography (TLC) and tested positively for the presence of alkaloids (Dragendorf reagent) and phenolic (ferric ion test) compounds. The chloroform fraction (950 mg) was submitted to normal-phase column chromatography. After combination of initial fractions based on TLC profiles, 10 fractions were obtained. Fraction 8 contained yellowish white crystals in the form of small plates or needles of 1 (24 mg). These procedures were partially described in Pohlit et al. (2012).

Spectral analysis and identification of 1 - ^{1}H, ^{13}C NMR and IR data obtained of the crystals obtained above were consistent with a structure containing a phenolic OH (δ_H 13.2; 3432 cm^{-1}) forming an intramolecular H-bond with the C=O of a xanthone (δ_C 181.1; 1608 cm^{-1}). Also, three singlets (CH_3O), a pair of doublets characteristic of *meta*-coupled aromatic Hs (J = 2 Hz) and a pair of doublets characteristic of *ortho*-coupled aromatic Hs (J = 9 Hz) were further evidence for a 1,3,7,8-tetrasubstituted xanthone. The EI-MS spectra of 1 exhibited ions m/z 302 [M^+] and 287 [(M^+-15), base peak]. Comparison of these data with ^{1}H and ^{13}C NMR spectral data in the literature (Tchamo et al. 2000, Dua et al. 2004) led to the identification of this compound as 1-hydroxy-3,7,8-trimethoxyxanthone whose trivial name is decussatin (1) (Fig. 1).

Extraction and isolation of 2 - Dried, powdered leaves (211 g) of *T. grandiflora* were macerated in ethanol (2 × 1 wk). After total evaporation, 49 g of the dry ethanol extract were dissolved in methanol-water (9:1) and this mixture was partitioned with hexanes. H_2O was added to the methanol-distilled water fraction to yield a 30% water solution, which was partitioned with chloroform. Hexanes fractions were combined, rotary evaporated and then freeze-dried to yield a dry, combined hexanes fraction (4.35 g, 8% w/w based on ethanol extract). A similar procedure applied to the chloroform fractions yielded a dry, combined chloroform fraction (13.62 g, 27.8%). Dry chloroform fraction (10 g) was dissolved in chloroform and chromatographed on a column of silica gel 60 (Merck, 0.063-0.200 mm, h × d = 10 × 7 cm). The column was eluted first with hexanes, then with a gradient of increasing polarity of acetone in chloroform. After combination based on TLC, five fractions resulted. Fraction 2 (2 g) was chromatographed on a column of silica gel 60 (h × d = 30 × 4.5 cm) using a gradient of increasing polarity

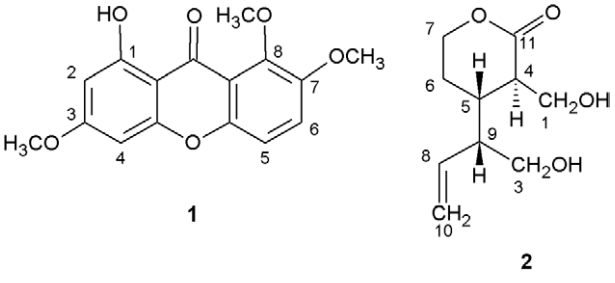

Fig. 1: decussatin (1-hydroxy-3,7,8-trimethoxyxanthone) (1) and amplexine (djalonenol, tetrahydro-3-(hydroxymethyl)-4-[1-(hydroxymethyl)-2-propenyl]-2H-pyran-2-one) (2) isolated from *Tachia grandiflora*.

of acetone in chloroform. After combining, nine fractions were obtained. Fraction 7 (323 mg) was separated by MPLC on a column of silica gel (h × d = 25.0 × 2.15 cm) using hexanes, then hexanes-chloroform-isopropanol (80:16:4) and finally chloroform-isopropanol (80:20). Six fractions were obtained and fraction 5 (101 mg) was separated on a column of Sepak silica (Varian, h × d = 30 × 2 cm) eluted with hexanes, then a gradient of hexanes-diethyl ether-isopropanol and finally isopropanol. Three fractions were obtained and fraction 3 (46 mg) was further purified by preparative TLC to yield 2 (25 mg) as a clear liquid [R_F = 0.60, ethyl acetate-methanol (2:1)] which, was identified as the secoiridoid monoterpene amplexine (or djalonenol) based on its spectral properties. This isolation procedure was partially described in Pohlit et al. (2012).

Spectral analysis and identification of 2 - From the ^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}H-COSY and HMQC spectra (in $CDCl_3$) of clear liquid 2 (isolated from the leaves of *T. grandiflora*), a terminal olefin was evidenced by coupled signals centered at δ 5.21 (2H, H-10a/H-10b) and δ 5.69 (1H, H-8) and ^{1}H-NMR and ^{1}H-^{1}H-COSY provided the connectivity through the following correlations: δ 5.69 (H-8) and δ 2.39 (H-9), δ 2.39 (H-9) and δ 3.62 (H-3a)/3.70 (H-3b), δ 2.39 (H-9) and δ 2.22 (H-5), δ 2.22 (H-5) and δ 2.75 (H-4)/δ 1.78 (H-6a)/δ 1.94 (H-6b), δ 1.78 (H-6a) and δ 1.94 (H-6b) and δ (H-7a)/δ 4.28 (H-7b). The presence of the lactone function was evidenced by signals at δ 175.0 (C-11) and δ 68.9 (C-7) in the ^{13}C-NMR spectra and by the intense infrared band at 1712 cm^{-1}. Using direct insertion on probe EI-MS, the MS spectrum of 2 ($C_{10}H_{16}O_4$, MW 200) exhibited a low intensity ion at *m/z* 201 [$(M+H)^+$] and ions at *m/z* 170 (loss of H_2C = O due to McLafferty Rearrangement) and *m/z* 152 (further loss of H_2O). Comparison of NMR and other data with literature data (Rasoanaivo et al. 1994, Onocha et al. 1995) allowed for the identification of compound 2 as amplexine [djalonenol, tetrahydro-3-(hydroxymethyl)-4 -[1-(hydroxymethyl)-2-propenyl]-2H-pyran-2-one].

Antimalarial activity - In vitro antiplasmodial assay - Chloroquine, pyrimethamine and cycloguanil-resistant *Plasmodium falciparum* K1 strain from the Center for Disease Control (CDC) (USA) was used to test antiplasmodial activity of extracts and fractions. *P. falciparum* W2-Indochina strain (CDC, USA) was used to test antiplasmodial activity of compound 2. The parasites were maintained in continuous culture at 37ºC using A+ blood cells in RPMI-1640 culture medium, which was enriched with 10% blood plasma (complete medium) and atmosphere of 5% CO_2, 5% O_2 and 90% N_2. Trophozoite-stages in sorbitol-synchronised blood (Lambros & Vanderberg 1979) were cultured at 1-2% parasitaemia and 2.5% haematocrit and then incubated with medium containing various concentrations of sample, either chloroquine diluted in culture medium - at standard concentrations (Rieckmann et al. 1978, WHO 2001) - or extracts, fractions (0.0032-50 µg mL^{-1}) and compound 2 (0.0005-50 µg/mL) in 10-fold serial dilutions were applied to microplate wells in triplicate. Negative (untreated) control wells having a 1% final concentration of dimethyl sulfoxide (DMSO) in culture medium were prepared in triplicate. The initial conditions of the test were adjusted so as to be similar to conditions used for parasite culture. The microplate was placed in an acrylic incubator where the mixture of gases used in culture was added and incubation occurred at 37ºC for 48 h. Then thin smears on microscope plates were prepared from each well and optical microscopy was used to quantify the number of surviving parasites per 2,000 red blood cells. Growth of parasites in contact with extracts, fractions and isolated substance was compared to that of negative controls and the inhibition of parasite growth was expressed as a percentage. The half-maximal inhibitory concentration (IC_{50}) responses were estimated by the probit method.

Cytotoxicity evaluation using the alamarBlue Assay - MRC-5 (human fibroblast) cells seeded in 96-well plates (10^4 cells per well) were treated with substances and the alamarBlue™ Assay was performed using the method of Ahmed et al. (1994). Briefly, after 24 h of culture, the extracts and fractions were individually dissolved in DMSO and added to each well (at well or final concentrations of 50 µg/mL) and incubated for 48 h. Doxorubicin (5 µg/mL) was used as positive control. Negative controls (blanks) received the same amount of DMSO and had the same final DMSO concentrations as samples (0.1%). Two hours before the end of the incubations, 10 µL of alamarBlue™ was added to each well. The fluorescent signal was monitored with a multiplate reader (DTX800, Beckman Coulter, Inc) using a 530-560 nm excitation wavelength range and 590 nm emission wavelength. The fluorescent signal generated from the assay was proportional to the number of living cells in the sample, according to the specifications of the manufacturer.

Statistical analysis - Data generated in the alamarBlue™ Assay are presented as mean ± standard error of the mean. The IC_{50} values and their 95% confidence intervals were obtained by nonlinear regression using the GRAPHPAD program (Intuitive Software for Science, San Diego, CA).

Animals and ethical approval - Adult Webster Swiss albino mice (26 ± 2 g weight) were used for the antimalarial and toxicity tests and received water and food *ad libitum*. In vivo tests were performed using Guidelines for Ethical Conduct in The Care and Use of Animals of Federal University of Rio Grande do Norte (CEUA 043/2010).

In vivo antiplasmodial activity - In vivo antimalarial activity was evaluated using *Plasmodium berghei* NK65 strain (drug-sensitive). This strain was maintained by successive passages of blood forms from mouse to mouse. The test protocol is based on the Peters 4-day suppressive test. Female Webster Swiss mice weighing 26 ± 2 g were used in this study. Animals were infected intraperitoneally with 0.2 mL of infected blood suspension containing 1 × 10^5 parasitised erythrocytes and randomly divided into groups of three individuals. Test groups were treated orally and subcutaneously at doses of 500 mg/kg/day of extracts. Positive control groups

received a dose of 10 mg chloroquine/kg/day orally or subcutaneously and negative control groups received 0.2 mL of 2% DMSO or saline. The animals were treated for four days starting 24 h after inoculation with *P. berghei*. On days 5 and 7 after parasite infection, blood smears were prepared from all mice, fixed with methanol, stained with Giemsa, then microscopically examined (1,000X magnification). Parasitaemia was determined in coded blood smears by randomly counting 2,000-4,000 erythrocytes in the case of low parasitaemias (≤ 10%) or up to 1,000 erythrocytes in the case of higher parasitaemias. Overall mortality was monitored daily in all groups during a period of four weeks following inoculation. The difference between the average parasitaemia of control groups (100%) and test groups was calculated as a percentage of parasite growth suppression (PGS) according to the equation PGS = 100 × (A – B)/A, where A is the average parasitaemia of the negative control group and B corresponds to the parasitaemia of the test group.

RESULTS

Chemistry - Since infusions of *T. grandiflora* are used in traditional medicine, polar extracts were prepared by hot extraction with water (infusion), hot continuous extraction with methanol in a soxhlet apparatus and room temperature maceration in ethanol. We detected alkaloids in the chloroform fractions obtained from the methanol extracts of roots, however, alkaloids were not isolated. Instead, needles or plates of 1 were isolated from the roots of *T. grandiflora* and a rare isolate 2 was isolated as a non-viscous, clear liquid, from the leaves of this plant. As detailed above, the identification of both substances was straight-forward based on spectral data and comparison with data in the literature for each compound.

Cytotoxicity to human fibroblasts - Extracts and fractions of *T. grandiflora* were evaluated for toxicity against human fibroblast cells (MCR-5) using the antitumor drug doxorubicin as positive control. At a concentration of 50 µg/mL, after 48 h of treatment, methanol and water extracts of roots, ethanol extracts of leaves, chloroform fractions of leaves and chloroform fractions of roots did not exhibit cytotoxicity to these normal human cells (IC_{50} > 50 µg/mL). Doxorubicin exhibited an IC_{50} of ca. 1.1 µg/mL against MCR-5 fibroblast cells.

In vitro antiplasmodial activity - The methanol and ethanol extracts of roots and leaves, respectively, exhibited no in vitro antiplasmodial activity against the K1 strain of *P. falciparum* (IC_{50} > 50 µg/mL). Liquid-liquid partitioning of these extracts provided chloroform fractions of the roots and the leaves exhibiting, respectively, moderate (IC_{50} = 10.5 µg/mL) and low (IC_{50} = 35.8 µg/mL) in vitro inhibitory activity against *P. falciparum* (Table I).

At different concentrations, amplexine (2) inhibited the in vitro growth of the blood forms of the human malaria parasite *P. falciparum* W-2 Indochina strain vs. untreated controls. Chloroquine was used as a positive control. From the dose-response curve it was possible to establish an IC_{50} of 7.1 µg/mL (Fig. 2).

In vivo antiplasmodial activity - Water extracts of the roots of *T. grandiflora* were the most active samples in vivo in *P. berghei*-infected mice in the Peters 4-day suppressive test. On day 5 after infection, water extract of the roots administered by mouth and subcutaneously was accompanied by the observation of 59% and 45% suppression, respectively, of *P. berghei* growth vs. untreated controls (Table II). Methanol extracts of the roots of *T. grandiflora* administered orally exhibited borderline moderate inhibitory activity (39% suppression of *P. berghei* growth) on the fifth day after infection in *P. berghei*-infected mice, but by the seventh day this effect had decreased substantially (Table II). Low in vivo activities (24-27% parasite suppression) were observed on day 5 for the methanol extracts of roots administered by subcutaneous injection and the ethanol extracts of leaves administered orally, but by day 7 the suppressive effects of these extracts was null. In general, mean survival times for *P. berghei*-infected mice which had received *T. grandiflora* extracts were equivalent to untreated controls (Table II).

TABLE I

In vitro median inhibition concentrations (IC_{50} ± standard deviation) of extracts and fractions of *Tachia grandiflora* against K1 strain of *Plasmodium falciparum*

Extract/fraction	*P. falciparum* IC_{50} (µg/mL)
Methanol extracts of roots	> 50.0
Chloroform fractions of roots	10.5
Ethanol extracts of leaves	> 50.0
Chloroform fractions of leaves	35.8
Water extracts of roots	> 50.0
Chloroquine diphosphate salt	0.13

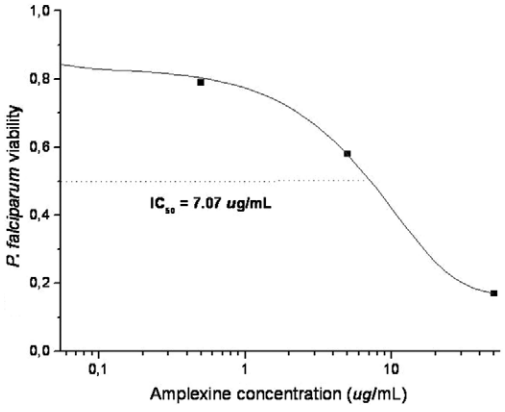

Fig. 2: dose-response curve for drug resistant *Plasmodium falciparum* W-2 strain in the presence of different concentrations of amplexine (djalonenol, tetrahydro-3-(hydroxymethyl)-4-[1-(hydroxymethyl)-2-propenyl]-2H-pyran-2-one) in a representative experiment. IC_{50}: half-maximal inhibitory concentration.

DISCUSSION

As the Krettli group found for the extracts of the roots of a *Tachia* sp. from the Brazilian Amazon (Carvalho et al. 1991, Brandão et al. 1992), we found that the extracts of roots and leaves of *T. grandiflora* studied herein were inactive (IC_{50} > 50 µg/mL) in vitro against *P. falciparum* (Table I). Through liquid-liquid partitioning of polar extracts, it was possible to obtain chloroform fractions of the roots and leaves. For the first time, in vitro antiplasmodial activity of a *Tachia* sp. against the human malaria parasite *P. falciparum* has been demonstrated (for these 2 chloroform fractions).

Little is known about the chemical composition of *Tachia* spp. Previously, Pio Corrêa (1926) described the isolation of a "bitter yellow glycoside (tachinine), bitter white, transparent crystalline plates (tachic acid) and rosettes of fine needles of a non-bitter alkaloid (caferanine)" from *caferana* (originally cited species: *T. guianensis*). The structures of these substances were not defined. Based on the diagnostic morphological characters of the drawing of *caferana* in Pio Correa's book, the species was either *Tachia lancisepala* or *Tachia siwertii*, both scientifically described only in 2005 (Struwe et al. 2005).

From the active root and leaf chloroform fractions of *T. grandiflora* the antiplasmodial tetra-oxygenated xanthone decussatin (1) and the rare seco-iridoid aglycone amplexine (2) were, respectively, isolated (Pohlit et al. 2012). Tetra-oxygenated xanthones like 1 are commonly isolated from gentians and seco-iridoids are also isolated from gentians. However, seco-iridoids generally occur as glycosides (Jensen & Schripsema 2002).

Decussatin (1), has been previously isolated from *Swertia alata* C.B. Clarke (Gentianaceae) and has been shown by Karan et al. (2005) to have an in vitro protective effect against malaria parasites. In general, hydroxyxanthones such as 1 act through a parasite specific mechanism of action by blocking haemozoin formation in the *P. falciparum* digestive vacuole through binding to haeme in soluble haeme-xanthone complexes (Riscoe et al. 2005).

In the present work, amplexine (2) isolated from the leaves of *T. grandiflora* inhibited the growth of the blood stages of the W-2 strain of *P. falciparum* in vitro. Amplexine (2) is the dihydroxy monoterpene aglycone of the secoiridoid 1-O-β-D-glucopyranosylamplexine that was first isolated from the leaves of *Anthocleista amplexicaulis* Baker (Gentianaceae) (Rasoanaivo et al. 1994) and later from the stems of *Anthocleista djalonensis* A. Chev. (Onocha et al. 1995). Interestingly, in Madagascar, a leaf decoction of *A. amplexicaulis* is used as a remedy for malaria (Rasoanaivo et al. 1994). This is the first report on the antiplasmodial activity of terpenoid 2. Interestingly, terpenes arrest *P. falciparum* development by inhibiting the biosynthesis of polyprenylated (terpenoid) primary metabolites such as dolichols, ubiquinones and menaquinones during the growth of *P. falciparum* parasites within infected red blood cells (Goulart et al. 2004). The presence of antiplasmodial compounds 1 and 2 in *T. grandiflora* is interesting given the widespread traditional use of this plant in the treatment of malaria in the Amazon Region.

The Krettli group collected a *Tachia* sp. (originally cited species: *T. guianensis*) in the Brazilian Amazon in São Félix do Xingú, state of Pará (Carvalho et al. 1991, Carvalho & Krettli 1991, Brandão et al. 1992, Krettli et al. 2001). Root infusions were prepared according to traditional practice and administered by mouth to *P. berghei*-infected mice at doses of 100, 500 and 1,000 mg/kg/day in the 4-day suppressive test. The inhibition obtained was ca. 40% vs. controls. Based on the known distributions of *Tachia* spp, it is likely that the plant studied by the Krettli group was either *T. grandiflora*, the very rare *T. siwertii* Struwe, Kinkade & Maas, or maybe even *Tachia occidentalis* Maguire and Weaver. The in vivo activity of *T. grandiflora* in *P. berghei*-infected mice herein, together with the Krettli et al.'s (2001) results points to the antimalarial potential of *caferana* (*Tachia* spp) infusions (Table II).

Extracts and isolated natural products that inhibit *P. falciparum* in vitro often may have little or no effect

TABLE II

In vivo suppression of *Plasmodium berghei* in infected mice and average mouse survival after oral and subcutaneous treatment with 500 mg/kg/day of extracts of *Tachia grandiflora* in the Peters 4-day suppressive test

	Parasite inhibition (%)				Average survival time ± SD	
	Oral		Subcutaneous			
Sample	Day 5	Day 7	Day 5	Day 7	Oral	Subcutaneous
Root water extract	59	24	45	0	24 ± 3	22 ± 2
Root methanol extract	39	11	27	0	21 ± 2	20 ± 3
Leaf ethanol extract	24	0	6	0	19 ± 3	22 ± 1
Chloroquine, 10 mg/kg/day	99	98	99	100	> 40	> 40
Control, blank	0	0	0	0	20 ± 5	22 ± 3

SD: standard deviation.

against *P. berghei* in vivo. This was observed for the isolated natural product 4-nerolidylcatechol that in general was active against *P. falciparum* strains in vitro, but only provided a suppressive effect on *P. berghei* in mice at high dose (600 mg/kg/day) (Rocha e Silva et al. 2011). On the other hand, the alkaloids ellipticine, olivacine and cryptolepine are active both in vitro against *P. falciparum* (IC_{50} = 0.35-1.4 μM) and in vivo against *P. berghei* in infected mice (10-100 mg/kg/day) (Rocha e Silva et al. 2012). The lack of in vitro antimalarial activity observed for polar extracts of *T. grandiflora* studied herein and the *Tachia* sp. studied by Krettli et al. (2001) mean that these extracts contain substances that mask, suppress or dilute the in vitro activity of antimalarial components that are present in these extracts and concentrated during the fractionation step that led to the preparation of the active chloroform fractions. Future work on the chemical composition of *Tachia* spp should feature an initial extraction step followed by liquid-liquid partitioning of the extract to generate simple fractions of increasing polarity that should be assayed both for in vivo activity against *P. berghei* and in vitro activity in a bioguided process.

Despite the affirmation in Pio Corrêa (1926) that *caferana* plants present toxicity. The extracts and fractions of roots and leaves of *T. grandiflora* were not toxic to human fibroblasts in vitro (IC_{50} > 50 μg/mL). Interestingly, Pohlit et al. (2007) found that fractions of leaf extracts of *T. grandiflora* were cytotoxic in vitro to five tumour cell lines (IC_{50} = 4.1-25.4 μg/mL). Thus, there is a selective in vitro cytotoxic effect of the leaf extracts and fractions against the faster growing tumour cells than against normal (human fibroblast) cells. Selective cytotoxicity and genotoxicity are common features of many drugs, including antimalarials such as artesunate and artemether. Mota et al. (2011) demonstrated that artesunate is cytotoxic and genotoxic to human lymphocytes and Alcântara et al. (2013) demonstrated that artemether was more cytotoxic to human lymphocytes than to gastric cancer cell line PG100. More studies on the in vitro cytotoxicity, mutagenicity and genotoxicity of *T. grandiflora* against different normal human cells and in vivo studies in animal models are needed to evaluate the potential pharmacological applications of *T. grandiflora* in humans.

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