

Quantification of Tagitinin C in *Tithonia diversifolia* by Reversed-phase High-performance Liquid Chromatography

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A simple, rapid and reliable reversed-phase high-performance liquid chromatographic method for the determination of tagitinin C, an anti-plasmodial sesquiterpene lactone isolated from the aerial parts of *Tithonia diversifolia*, has been developed. The assay has been used to quantify tagitinin C in various extracts of the aerial parts of *T. diversifolia*. Copyright © 2003 John Wiley & Sons, Ltd.

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INTRODUCTION

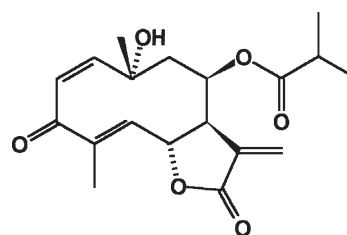
Tithonia diversifolia (Hemsley) A. Gray (family Asteraceae) is a shrub which is native to Central America and has become naturalised throughout the tropics (Pereira *et al.*, 1997). The aerial parts are traditionally used in the form of a decoction for the treatment of malaria in the islands of São Tomé and Príncipe in the Gulf of Guinea (do Céu de Madureira *et al.*, 2002). The main anti-plasmodial activity of this plant has been attributed to the sesquiterpene lactone, tagitinin C (**1**) (Goffin *et al.*, 2002), originally found in *T. tagitiflora* Desf. (Pal *et al.*, 1977), which exhibits significant activity against *Plasmodium falciparum* with an IC₅₀ of 0.95 µM (chloroquine-sensitive strain FCA) and 0.72 µM (chloroquine-resistant strain FCB₁).

The presence of tagitinin C in *T. diversifolia* varies considerably according to the source of the plant material. Thus, whilst **1** has been found in Indian plants (Baruah *et al.*, 1979; Bordoloi *et al.*, 1996), the sesquiterpene lactone has not been described in plants growing in Brazil (Pereira *et al.*, 1997), Costa Rica (Schuster *et al.*, 1992) and Taiwan (Kuo and Chen, 1998). As tagitinin C is easily detectable under UV (at 254 nm), different chemical races of *T. diversifolia* with specific geographic distribution might exist. However, no facile assay has been published for the accurate determination of the amount of **1** in samples of *T. diversifolia* or any other plant species. Consequently, the development of an HPLC method for the rapid quantification of tagitinin C in this species, in which the qualitative and quantitative composition appears very variable, was considered desirable.

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Tagitinin C (**1**)

EXPERIMENTAL

Materials. Aerial parts of *Tithonia diversifolia* (Hemsley) A. Gray were collected in November 1997 in the São Tomé and Príncipe islands. A voucher specimen (MM 626) of the plant material has been deposited at the Botanic Institute of the University of Coimbra, Portugal. Additional samples of *T. diversifolia* used in the analytical study were collected in São Tomé and Príncipe in July 1998. Samples of tagitinin C used for the quantification study were isolated in our laboratory from *T. diversifolia*. Identification of **1** was established by spectroscopic methods according to the literature (Pal *et al.*, 1977; Baruah *et al.*, 1979) and the purity (97.2%) of the sesquiterpene lactone was calculated by the normalisation procedure. All chemicals used were of HPLC grade and purchased from VWR International (Darmstadt, Germany).

Extraction of samples. Dried aerial parts (500 mg) of *T. diversifolia* were powdered and extracted with diethyl ether (40 mL) at room temperature for 2 h with constant stirring. The mixture was filtered, the residue extracted twice more with diethyl ether (20 mL), and the ether extracts combined and evaporated to dryness under reduce pressure. A similar protocol was employed to obtain methanol and dichloromethane extracts of the

dried plant material. A decoction of the aerial parts (500 mg) of the plant was also prepared and lyophilised. Dry extracts (8 mg) were redissolved in a mixture of 9 mL acetonitrile and 11 mL acetate buffer (pH 4.8) and the solutions were filtered through a 0.45 µm membrane HVLP filter prior to HPLC analysis.

Chromatographic conditions. An LKB (Bromma, Sweden) 2249-010 LC gradient pump equipped with a Hewlett Packard (Palo Alto, CA, USA) model 1040 M-series 2 photodiode-array detector operating at 254 nm was employed. The column was a Lichrospher 60 RP Select B (250 × 4 mm i.d.; particle size 5 µm; VWR International), and the mobile phases were acetonitrile (solvent A), and aqueous sodium acetate (0.1 M) adjusted to pH 4.8 with acetic acid (10%) (solvent B). Elution was isocratic with A:B (45:55) at a flow rate of 1.0 mL/min. Pure tagitinin C eluted at ca. 5.8 min on this system.

Quantification of tagitinin C. The analysis of tagitinin C was carried out using the external standard method. Working solutions containing 0.4, 0.2, 0.1 and 0.01 mg/mL of **1** were prepared from a stock solution of the sesquiterpene lactone (1 mg/mL) in the mobile phase. Triplicate injections (20 µL) at each concentration were run and the mean peak area, detected at 254 nm, was used to establish the calibration curve. In order to validate the method, three separate determinations of the standard curve were performed. For each concentration, the data collected from the three determinations were averaged and re-plotted to yield the composite standard curve for **1** (linear regression equation: $y = 19,329.09x - 18.50$; $r^2 = 0.99936$).

For each sample of *T. diversifolia*, an aliquot (20 µL) of the extract was injected into the HPLC system and the peak area associated with **1** was integrated and used to calculate the amount of tagitinin C by applying the composite linear regression equation. In order to determine the efficiency of the diethyl ether extraction procedure, pure **1** (1.5 mg and 3 mg) was added to 500 mg of powdered aerial parts of *T. diversifolia* prior to extraction and the complete assay procedure was carried out in triplicate.

RESULTS AND DISCUSSION

HPLC chromatograms of diethyl ether extracts of the aerial parts of *T. diversifolia* obtained using the procedure described exhibited good baseline stability, high resolution and short analysis time for tagitinin C (Fig. 1). In order to demonstrate the repeatability of the HPLC method, the amount of **1** was determined in six ether extracts of the aerial parts of the plant and a value of 30.5 ± 2.1 as the percentage of the sesquiterpene lactone in the ether extract was obtained.

To test the efficiency of extraction, standard **1** was added to 500 mg of powdered plant material prior to extraction. The peak corresponding to tagitinin C was found to increase proportionally with the increased amount of additive. The recovery from the plant matrix was estimated to be $99.3 \pm 0.8\%$ (1.5 mg of **1** added) and $102.5 \pm 0.2\%$ (3 mg of **1** added). This, together with evidence of peak purity as determined by spectral matching, indicates that the method shows adequately accuracy.

Different extracts of *T. diversifolia* were prepared and analysed as described. Tagitinin C was found mainly in the diethyl ether extracts of leaves and flowers (Table 1); the ether extracts of stems, and the dichloromethane, methanol and water extracts of aerial parts showed considerably lower values of **1**. Clearly leaf material is the most appropriate part of *T. diversifolia* for the extraction of tagitinin C in highest yield.

As the aerial parts of *T. diversifolia* appear to contain very variable amounts of tagitinin C according to the geographical location of the source material and its genetic make-up, quantification of this sesquiterpene lactone is essential in order to select those chemical races of the plant showing the highest anti-plasmodial activities. The simple and reliable chromatographic quantification procedure developed here may be easily adapted to determine **1** in other sample matrices, e.g. in extracts of *T. tagitiflora*, and will also be valuable in the standardisation of derived products used to treat malaria.

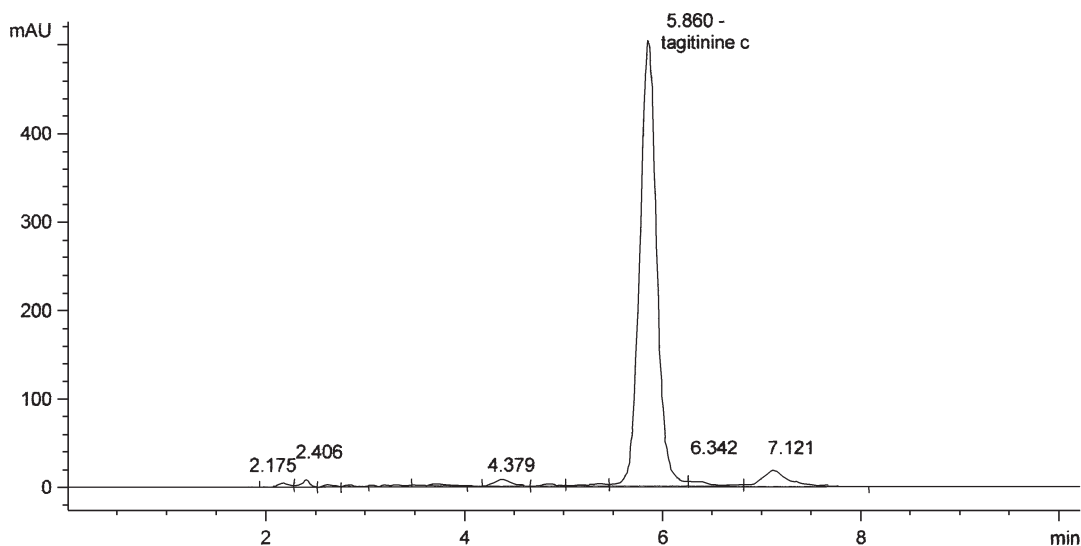


Figure 1. Typical reversed-phase HPLC chromatogram (detected at 254 nm) of a diethyl ether extract of the aerial parts of *Tithonia diversifolia* (for extraction and chromatographic protocols see Experimental section).

Table 1. Determination by reversed-phase HPLC of the amount of tagitinin C in various extracts of the aerial parts of *Tithonia diversifolia*

Part of plant assayed	Month of collection	Extraction solvent	Tagitinin C in extract ^a	Weight of extract (mg)	Tagitinin C in dried plant ^a
Aerial parts	November	Diethyl ether	30.53 ± 0.65	12.5	0.763 ± 0.016
Aerial parts	July	Diethyl ether	31.30 ± 1.53	12.5	0.782 ± 0.038
Aerial parts	November	Dichloromethane	0.492 ± 0.015	13.2	0.013 ± 0.001
Aerial parts	November	Methanol	1.987 ± 0.012	151.0	0.600 ± 0.004
Aerial parts	November	Water (decoction)	0.492 ± 0.015	91.5	0.090 ± 0.003
Flowers	November	Diethyl ether	5.080 ± 0.030	12.5	0.127 ± 0.001
Leaves	November	Diethyl ether	30.85 ± 0.31	18.6	1.145 ± 0.012
Stems	November	Diethyl ether	0.060 ± 0.005	2.9	0.00035 ± 0.00003

^a Values represent percentages (w/w); mean values ($n = 3$) ± standard deviation.

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