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FT–IR measurement of tagitinin C after solvent extraction from *Tithonia diversifolia*

E. Ziémons [a,*], E. Goffin [b], R. Lejeune [a], L. Angenot [b], L. Thunus [a]

[a] *Laboratory of Analytical Chemistry, Bioanalytical and Biopharmaceutical Research Center, Institute of Pharmacy, University of Liège (ULg), CHU, Tour 4, Avenue de l'Hôpital 1, Liège B-4000, Belgium*
[b] *Laboratory of Pharmacognosy, Natural and Synthetic Drugs Research Center, Institute of Pharmacy, University of Liège (ULg), CHU, Tour 4, Avenue de l'Hôpital 1, Liège B-4000, Belgium*

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Abstract

Tagitinin C, an antiplasmodial compound, identified as one major compound of the subtropical medicinal plant, *Tithonia diversifolia*, was determined by FT–IR spectroscopy method. The crude ether extracts from aerial parts of the plant were evaporated to dryness and re-dissolved in tetrachloroethylene (C_2Cl_4) before analysis.

The magnitude of the absorbance of the very specific C=O stretching vibration ($\nu_{C=O}$) at 1664.8 cm^{-1} was exploited in order to quantify tagitinin C. The determination coefficient (r^2) of the calibration scale was 0.9994, the detection limit was lower than 3 $\mu g\,ml^{-1}$ and the quantification limit was lower than 10 $\mu g\,ml^{-1}$.

Recovery values from 100.5 to 101.7% were found for spiked concentration levels from 19.91 to 89.95 $\mu g\,ml^{-1}$. The main characteristics of the curves obtained from the calibration standards and from the standard addition technique were not statistically different (Student *t*-test) suggesting that matrix effects were negligible.

The results obtained for the determination of tagitinin C in the crude ether extract from aerial parts of *T. diversifolia* by LC and FT–IR spectroscopic method agreed well: 0.76 ± 0.02 and 0.773 ± 0.009, of tagitinin C in dried plant respectively.
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1. Introduction

Malaria is by far the world's most important tropical parasitic disease and one of the top three killers among communicable diseases.

Worldwide prevalence of the disease is estimated to be in the order of 300–500 million clinical cases each year and mortality due to malaria is estimated to be over 1 million deaths, mostly children, each year according to an estimation of the World Health Organisation [1,2].

The causative agents in humans are four species of *Plasmodium: P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. Of these, *P. falciparum* accounts for the majority of infections and is the most lethal. The emergence of multi-drug resistant strains of *P. falciparum* underlines the importance to investigate new antimalarial compounds.

Tagitinin C was isolated from the aerial parts of the plant *T. diversifolia* (Asteraceae) and showed antiplasmodial activity against *P. falciparum* [3]. This compound is a sesquiterpene lactone (Fig. 1) and its structure was determined by comparison of spectral properties (UV, IR, ^{1}H NMR, ^{13}C NMR, ESI mass and optical rotation data) with previously reported values [4,5]. Nevertheless, very few data were found in the literature on the analytical determination of Tagitinin C in the plant. Recently, Goffin et al. described a LC method for the determination of tagitinin C in the aerial parts of Tithonia diversifolia (Hemsley) A. Gray [6].

Quantification of organic compounds in complex matrices such as plant materials [7,8], pesticide formulations [9,10], capsules [11], creams and ointments [12], by FT–IR spectroscopy with good accuracy and reproducibility has been carried out in solution.

In FT–IR, the C=O stretching vibration ($\nu_{C=O}$) provides the most characteristic band of carbonyl compounds (ketones, aldehydes, esters, lactones, ...). Tagitinin C has a

* Corresponding author. Fax: +32-4-3664324.
E-mail address: eziemons@ulg.ac.be (E. Ziémons).

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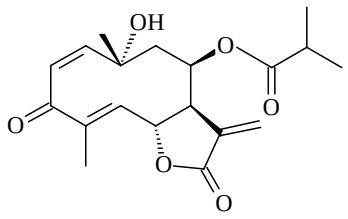

Fig. 1. Structure of tagitinin C.

particular group with the unsaturated ketone. This structure is not often found in plants. Pereira et al. describe sesquiterpene lactones in the aerial parts of Brazilian *T. diversifolia* [13]. An artemisinic acid analogue compound has been isolated from mature stems of Indian *T. diversifolia* [14]. The absence of the unsaturated ketone $\nu_{C=O}$ in other described sesquiterpene lactones is particularly interesting in that it could allow to quantify the tagitinin C in extracts of *T. diversifolia*.

The aim of this work was the development of a procedure without any purification steps for the determination of tagitinin C in the aerial parts of *T. diversifolia* using liquid extraction with ether and FT–IR measurements.

2. Experimental

2.1. Apparatus and reagents

A Perkin-Elmer Spectrum GX® FT-IR (Perkin-Elmer Limited, Beaconsfield, England), equipped with a MIRTGS detector, was employed for spectral measurements. The sample compartment was equipped with a shuttle with two places.

Solutions in tetrachloroethylene were scanned in a liquid transmission cell provided with sodium chloride windows. This cell had an internal volume of 200 μl and a pathlength of 500 μm.

Spectra treatment and data manipulation have been carried out using Spectrum V2.00 software from Perkin-Elmer.

Uvasol® tetrachloroethylene (C_2Cl_4), diethyl ether (p.a.) and LiChrosolv® acetonitrile were obtained from Merck (Darmstadt, Germany).

The Bransonic® ultrasonic water bath was obtained from Branson Ultrasonics B.V. (Soest, the Netherlands).

Unless otherwise specified, solutions were filtered through a poly(vinylidene difluoride) (PVDF) membrane (pore size 0.45 μm) from Millipore (Bedford, MA).

The tagitinin C, isolated and purified by Goffin et al. [3] was employed for standard preparation. Its purity (97.2%) was determined by LC normalization procedure.

The LC system consisted of a LKB 2249-010 Gradient pump (LKB, Bromma, Sweden) equipped with a diode-array detector model HP 1040 M-series 2 (Hewlett-Packard, Palo Alto, USA) operating at 250.4 nm. The column was a Lichrospher 60 RP Select B (250 mm × 4 mm i.d.; particle size of 5 μm) obtained from Merck (Darmstadt, Germany). The mobile phase were: solvent A, acetonitrile; solvent B, aqueous solution of sodium acetate (0.1 M) adjusted to pH 4.8 with acetic acid (10%). Elution was isocratic with A:B (45:55) at a flow rate of 1.0 ml min^{-1}. Extracts, dissolved in a mixture of acetronitrile and acetate buffer (pH 4.8) (9:11), were introduced using a 20 μl loop valve [6].

2.2. Plant material

The aerial parts of *T. diversifolia* (Hemsley) A. Gray (Asteraceae), collected at the Democratic Republic of São Tomé e Principe in November 1997, were provided by Professor A. Proença da Cunha (University of Coimbra, Portugal).

A voucher specimen (MM 626) has been deposited at the Botanic Institute of the University of Coimbra. The moisture content was 8.19 ± 0.02 wt.%.

All sample plants were thoroughly ground and sieved to 250 μm size in an Ultra Centrifugal Mills ZM 100 (Retsch, Germany).

2.3. Procedure

The dried aerial parts (500 mg) of *T. diversifolia* were powdered and extracted three times. First, the powder was stirred with 40 ml ether at room temperature for 2 h and the liquid phase was filtered through a filter paper 604 (Schleicher & Schüll, Dassel, Germany). Second, the filtered powder was extracted with 20 ml ether under conditions described above. Third, the filtered powder was extracted once again with 20 ml under conditions described above. The three ether extracts were bulked in a 100 ml marked flask and this flask was filled with ether. Subsequently 10 ml of ether solution was evaporated to dryness under reduced pressure. The residue was solubilized in 1 ml of C_2Cl_4 using ultrasonic shaking during 5 min in an ultrasonic water bath and analyzed by FT–IR (solution I). Spectra were the average of eight co-added scans collected in the shuttle mode from 4000 to 1150 cm^{-1} at 4 cm^{-1} resolution and 0.5 cm^{-1} intervals.

A strong Norton–Beer apodization function was used to give the best compromise between reduction of slidelobes and increase in spectral bandwidth. The shuttle mode allows sample to be moved in and out of infrared beam for automatic background correction during sample measurement (4 backgrounds/8 scans/4 backgrounds). The spectrum of C_2Cl_4 was registered under the described conditions and was stored as the reference solvent spectrum. The spectrum of the sample was registered under the described conditions. The spectrum of the extract was obtained by subtraction of the reference solvent spectrum from the sample spectrum.

A decoction was also made. The dried aerial parts (500 mg) were extracted with 20 ml distilled boiling water

for 15 min. The aqueous extract was lyophilized and solubilized in 1 ml of C_2Cl_4, using ultrasonic shaking during 5 min in an ultrasonic water bath and the solution was filtered. The aqueous extract was analyzed by the FT–IR method described above.

The band at 1664.8 cm^{-1} due to the unsaturated ketone was selected for analytical measurements using a baseline correction between 1685 and 1642 cm^{-1} (both sides of this band). Its absorbance was compared to those of calibration standards to quantify tagitinin C.

C_2Cl_4 solutions of tagitinin C (50–150 $\mu g\,ml^{-1}$), measured in the same way as samples, were employed as standards.

3. Results and discussion

Fig. 2 shows the FT–IR spectra of solution I and a standard solution of tagitinin C. As it can be seen, well-defined absorbance spectra were obtained between 4000 and 1150 cm^{-1} using C_2Cl_4.

Solution I and standards have intense bands in the wavenumber range from 1820 to 1600 cm^{-1}. The spectrum of tagitinin C in C_2Cl_4 presents three bands at 1664.8, 1741.3 and 1779.6 cm^{-1}. According to its structure, it is reasonable to think that these peaks correspond to the $\nu_{C=O}$ of unsaturated ketone (dienone), ester and lactone, respectively.

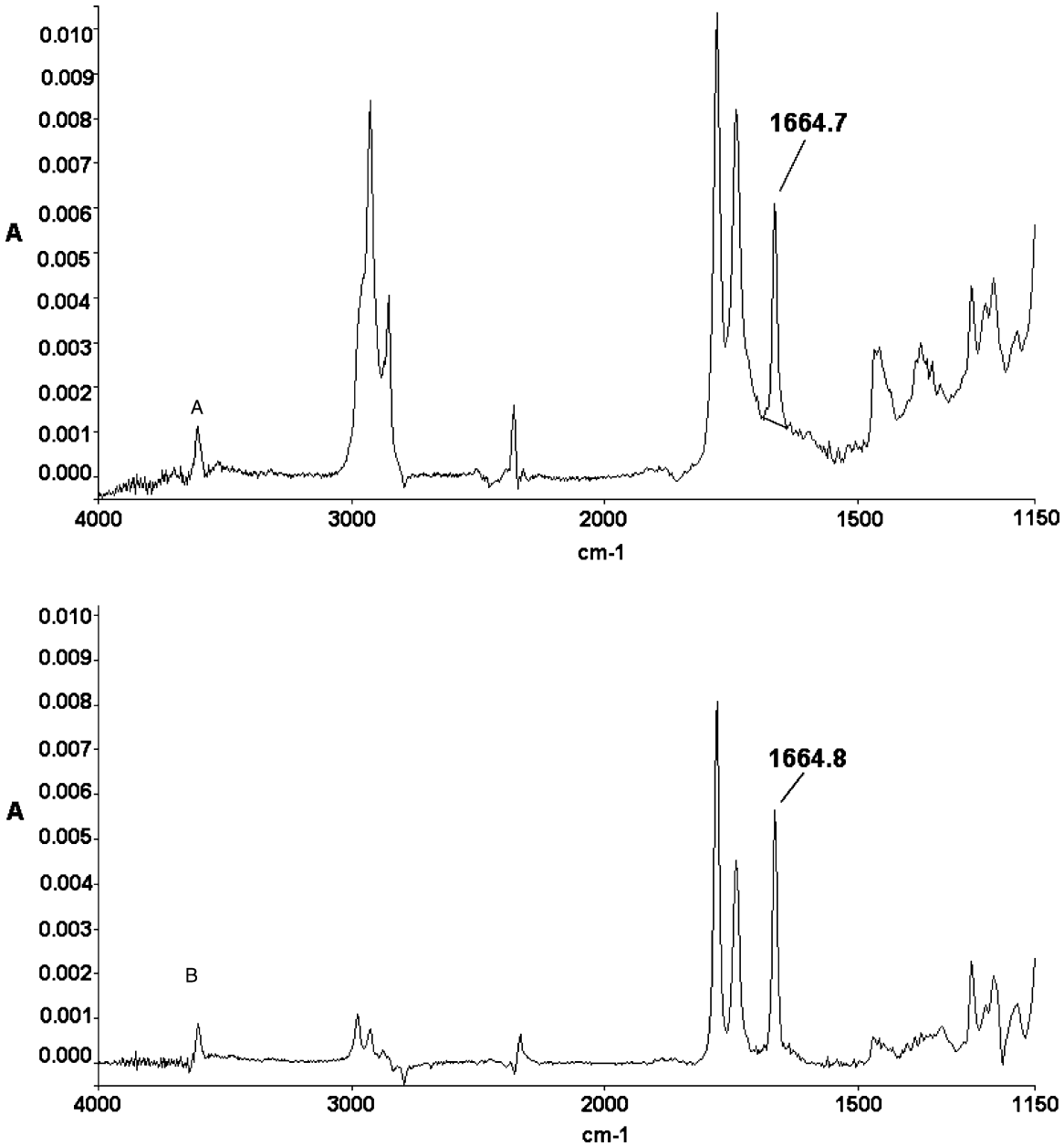

Fig. 2. (A) Infrared spectrum of the solution I containing 53.31 $\mu g\,ml^{-1}$ of tagitinin C. (B) Infrared spectrum of tagitinin C (50 $\mu g\,ml^{-1}$ C_2Cl_4).

On the solution I spectrum of *T. diversifolia* in C_2Cl_4 are also found the $\nu_{C=O}$ corresponding to an unsaturated ketone at 1664.7 cm^{-1}, an ester at 1740.6 cm^{-1} and a lactone at 1779.0 cm^{-1} (Fig. 2). The ratio between unsaturated ketone, ester and lactone bands intensity is different from the ratio obtained from tagitinin C. It stands to reason that the extract consists in a mixture of unidentified products strongly absorbing between 1800 and 1700 cm^{-1} and tagitinin C present as a main product. As one can also see, a residual absorbance modifies the baseline around the signal at 1664.8 cm^{-1}. Therefore, to be able to correctly determine the maximum height of this $\nu_{C=O}$, it is important to take into account the baseline deviation and to integrate it using a correction baseline joining the two sides of the peak. This baseline starts at 1685 and stops at 1642 cm^{-1}.

3.1. Validation of the FT–IR spectroscopic method

To evaluate the possible interference of the *T. diversifolia* matrix on the FT–IR determination, several statistical tests were realized on data obtained using C_2Cl_4 solutions of tagitinin C and technique of standard addition on solution I.

The calibration curve linearity for C_2Cl_4 solutions of tagitinin C was investigated with five calibration standards (50, 75, 100, 125, 150 $\mu g\,ml^{-1}$ C_2Cl_4) carried out in triplicate, run on three different days. The absorbance at 1664.8 cm^{-1} was used. These results are shown in the first column of Table 1. A good determination coefficient was found and each statistical test was passed. Thus, it results that the absorbance is proportional to the concentration of tagitinin C.

On the other hand, the calibration curve linearity of the standard addition technique was performed using solution I spiked with known amounts of tagitinin C (19.91, 55.17 and 89.95 $\mu g\,ml^{-1}$) carried out in triplicate. The absorbance at 1664.8 cm^{-1} was also used. The results displayed in the second column of Table 1 were obtained with the corrected equation. This one is calculated by the subtraction of the coefficient *a* obtained by the multiple linear regression ($y = bx + a$) from the absorbance of each added amounts of tagitinin C. This mathematical transformation allows to perform the intercept test. A good determination coefficient was also found and each statistical test was also passed. These results also indicated that the absorbance is proportional to the added amounts of tagitinin C.

Table 1
Linearity between the tagitinin C concentration and the absorbance at 1664.8 cm^{-1} for standard solutions and standard addition technique

Parameters	Standard solutions	Standard addition technique
Determination coefficient	0.9994	0.9998
Cochran test [15]—Homoscedasticity (g)	0.4482	0.6884
Reference value (0.95)	0.6838	0.7679
Significative slope test	21992	66579
Reference value (0.95)	4.67	4.96
Intercept test	1.22	0
Reference value	2.16	2.23
Validity test for the calibration linearity	1.85	2.73
Reference value	3.71	4.46
Limit of detection ($\mu g\,ml^{-1}$) [16]	2.77	1.48
Limit of quantification ($\mu g\,ml^{-1}$) [16]	9.23	4.61

Table 2
Fidelity of the method for standard solutions

	Tagitinin C ($\mu g\,ml^{-1}$)	Coefficient of variation (%) at 1664.8 cm^{-1}
Repeatability ($n = 3$)	50	1.19
	100	0.53
	150	0.54
Reproducibility ($n = 3$)	50	2.81
	100	0.53
	150	0.69

The variation coefficient obtained in the Table 2 shows the fidelity of the method for standard solutions.

The accuracy of the method is demonstrated by the fact that the true value (100% of each concentration) is included between the confidence interval (Table 3).

Moreover, both slopes were also compared in order to determine the influence of matrix on the signal at 1664.8 cm^{-1}. The regression line for the standard solutions was: $A = 8.04 \times 10^{-5} + 9.20 \times 10^{-5}$ (sb $= 6.21 \times 10^{-7}$) C. This equation compares well with that obtained by solution I spiked with known amounts of tagitinin C, which was equal to $A = 0.004833 + 9.25 \times 10^{-5}$ (sb $= 3.58 \times 10^{-7}$) C. According to Student *t*-test, both slopes are similar and thus these results strongly suggest the absence of matrix effect on FT–IR method. We also used the two equations to calculate the concentration of solution I. Refering to the equation obtained with standard solutions, the obtained concentration was equal to $53.3 \pm 0.9\,\mu g\,ml^{-1}$ and using the equation obtained by the standard addition technique, the concentration was equal to $52.2 \pm 0.2\,\mu g\,ml^{-1}$. These values are similar and confirm the Student *t*-test data.

In order to evaluate the accuracy of the developed procedure the study of the percentage recovery in crude ether extracts spiked with known amounts of tagitinin C was realized. The results are summarized in Table 4.

Table 3
Accuracy of standard solutions and standard addition methods

Standard solutions		Standard addition technique	
Tagitinin C ($\mu g\,ml^{-1}$)	Confidence interval	Tagitinin C added ($\mu g\,ml^{-1}$)	Confidence interval
50	99.48–103.05	19.91	98.11–100.77
100	99.02–100.68	55.17	99.54–102.10
150	98.91–101.10	89.95	99.02–100.68

Table 4
FT–IR determination of recovery of tagitinin C added to solution I containing 53.3 ± 0.9 μg ml^{-1} of tagitinin C

Tagitinin C added (μg ml^{-1})	Tagitinin C in spiked crude ether extracts (μg ml^{-1})	Tagitinin C obtained (μg ml^{-1})	Recovery (%)
19.91	73.3 ± 0.2	20.0 ± 0.2	100.5 ± 0.7
55.17	109.2 ± 0.3	55.9 ± 0.3	101.4 ± 0.5
89.95	144.8 ± 0.3	91.5 ± 0.3	101.7 ± 0.3

Each measurement was carried out in triplicate.

Table 5
Comparison of FT–IR and LC methods

Extract	Tagitinin C % in dried plant	
	FT–IR method (at 1664.8 cm^{-1})	HPLC method [6]
Ether (collecting date: November 1997)	0.773 ± 0.009	0.76 ± 0.02
Ether (collecting date: July 1998)	0.758 ± 0.004	0.78 ± 0.04
Decoction (collecting date: November 1997)	Not detected	Not detected

Each measurement was carried out in triplicate.

Recovery values varied from 100.5 to 101.7%, thus indicating the applicability of this simple procedure to obtain accurate results.

A comparative investigation with a LC method [6] was achieved on dried plants collected in November 1997 and July 1998. This LC method was also characterized by good linearity. The regression line was: $A = -18.50 + 19329.09\,C$ with $r^2 = 0.9994$ in the concentration range for C comprised between 10 and 400 μg ml^{-1}.

Table 5 shows that our results obtained by FT–IR spectroscopy are comparable with data obtained by the LC method.

We also applied the FT–IR method to decoction extracts for the quantification of tagitinin C in dried aerial parts of *T. diversifolia* because this extraction method is frequently used to obtain herbal remedies. The results are also displayed in Table 5 with data obtained by the LC method. Unfortunately, this simple extraction procedure does not allow to extract tagitinin C from the aerial parts of *Tithonia diversifolia*.

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References

[1] WHO, World Malaria situation revised in October 1998, 2002.
[2] J. Sachs, P. Malaney, Nature 415 (2002) 680.
[3] E. Goffin, E. Ziemons, P.M. De Mol, M. de Madureira, A.P. Martins, A. Proença da Cunha, G. Philippe, M. Tits, L. Angenot, M. Frederich, Planta Med. 68 (2002) 543.
[4] R. Pal, D.K. Kulshreshtha, R.P. Rastogi, Indian J. Chem. 15 (1977) 208.
[5] N.C. Baruah, R.P. Sharma, K.P. Madhusudanan, G. Thyagarajan, J. Org. Chem. 44 (1979) 1831.
[6] E. Goffin, A. Proença da Cunha, E. Ziemons, M. Tits, L. Angenot, M. Frederich, Phytochem. Anal., in press.
[7] E. Bloszyk, B. Geppert, B. Drozdz, Planta Med. 34 (1978) 79.
[8] J.M. Garrigues, M. Akssira, F.J. Rambla, S. Garrigues, M. de la Guardia, Talanta 51 (2000) 247.
[9] S. Armenta, G. Quintas, J. Moros, S. Garrigues, M. de la Guardia, Anal. Chim. Acta 468 (2002) 81.
[10] G. Quintas, A. Morales-Noé, C. Parrilla, S. Garrigues, M. de la Guardia, Vib. Spectrosc. 31 (2003) 63.
[11] X. Otte, R. Lejeune, L. Thunus, Anal. Chim. Acta 355 (1997) 7.
[12] X. Otte, B. Evrard, L. Delattre, L. Thunus, Anal. Chim. Acta 451 (2002) 323.
[13] P.S. Pereira, D.A. Dias, W. Vichnewski, A.M. Turco Tucci Nasi, W. Herz, Phytochemistry 45 (1997) 1445.
[14] M. Bordoloi, N.C. Barua, A.C. Ghosh, Phytochemistry 41 (1996) 557.
[15] W.G. Cochran, J. Roy. Stat. Soc. 4 (1937) 102.
[16] I.C.H., Guidance for Industry: Q2B Validation of Analytical Procedures: Methodology, 1996.