SUPERCRITICAL FLUID EXTRACTION OF TAGITININ C FROM *TITHONIA DIVERSIFOLIA*: COMPARISON OF EXTRACTION YIELD AND SELECTIVITY BETWEEN SUPERCRITICAL FLUID AND CLASSICAL METHODS OF EXTRACTION

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ABSTRACT

Tagitinin C was extracted from the aerial parts of *Tithonia diversifolia* using supercritical carbon dioxide and was quantified by FTIR spectroscopy.

The optimized supercritical fluid extraction (SFE $_{optimized}$) was compared to Soxhlet extraction with dichloromethane (S) and to maceration followed by lixiviation with ether (ML). The results demonstrated that the SFE $_{optimized}$ is an effective and selective extraction method for tagitinin C. Soxhlet extraction with dichloromethane and maceration followed by lixiviation with ether gave similar extraction yields but the tagitinin C concentration in S extract (15.6% w/w) and in ML extract (30.7% w/w) was lower than that in the optimized SFE extract (52.8% w/w).

INTRODUCTION

Tithonia diversifolia (Asteraceae) is a shrub which is native to Middle America and the West Indies. This plant has become naturalized around the tropics. Its aerial parts are traditionally used for the treatment of malaria in the Democratic Republic of Sao Tomé e Principe. Goffin et al. [1] isolated the tagitinin C (Fig. 1), a known sesquiterpene lactone, from the aerial parts of the plant and discovered its antimalarial activity against *P. falciparum*. Jian-Qiao Gu et al. recently showed significant antiproliferative activity of tagitinin C [2].

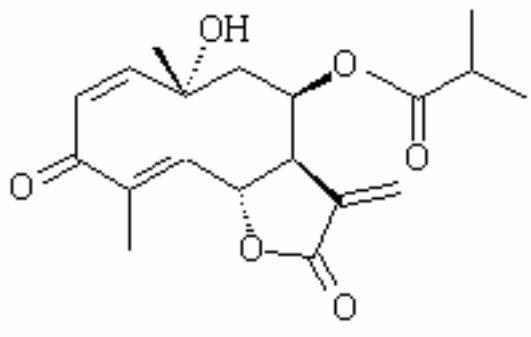

Figure 1 : Structure of tagitinin C

Supercritical fluid extraction is known as efficient method for the extraction of non polar compounds from plant matrices. Carbon dioxide is the most widely used solvent for extraction of natural products for foods and medicines, under mild conditions. It is inert, inexpensive, odourless, tasteless and environment-friendly solvent. Further, there is no solvent residue in the extract, since it is a gas in the ambient condition [3] [4].

Several sesquiterpene lactones, such as santonin [5], parthenolide [6], costunolide [7] and artemisinin [8,9] were already extracted by SFE with good results.

The aim of this work is to compare the extraction yield of tagitinin C and the selectivity of the carbon dioxide supercritical fluid extraction to classical extraction methods as Soxhlet extraction (S) with dichloromethane and maceration followed by lixiviation with ether (ML).

MATERIALS AND METHODS

1. Materials

All solvents used were of analytical grade.

The carbon dioxide, 99.98 % (w/w), was purchased from Air liquide (Liège, Belgium).

The aerial parts of *T. diversifolia* were provided by Professor A. Proença da Cunha (University of Coimbra, Portugal). 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).

The tagitinin C, isolated and purified by Goffin et al. was employed for standard preparation. Its purity (97.2%) was determined by HPLC normalization procedure [10].

2. Supercritical fluid extraction

Supercritical fluid extractions were performed using a Varian Star SFE Autroprep 44 (Suprex Corporation, Blacksburg-VA, USA). Figure 2 gives the flow sheet of supercritical CO_2 extraction apparatus.

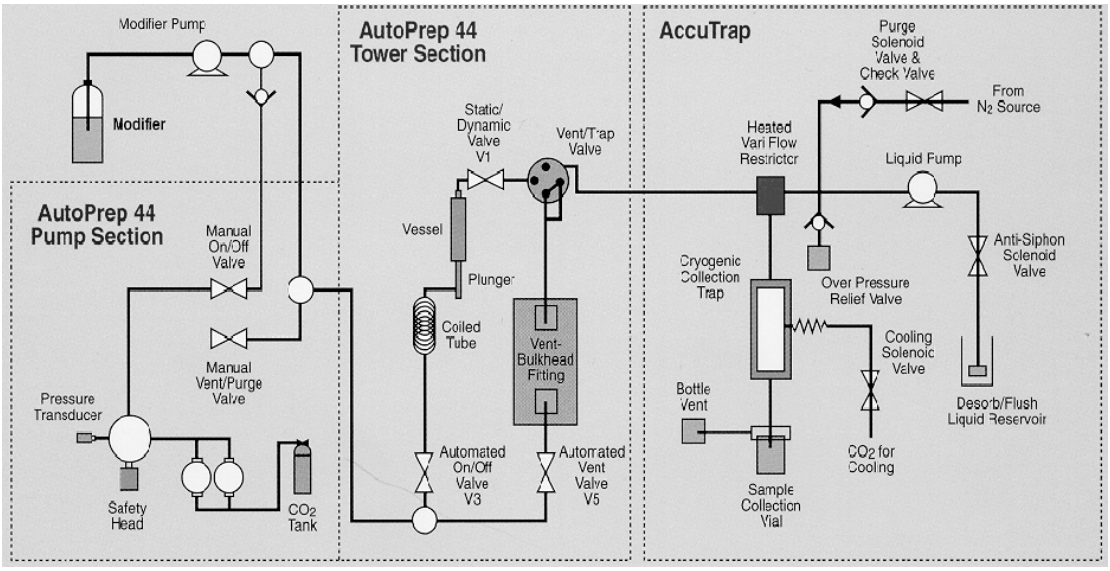

Figure 2: Schematic diagram of supercritical CO_2 extraction apparatus.

The extraction vessel (1mL) was packed with 200 mg of plant material. The flow rate and the amount of carbon dioxide were fixed at 1 mL min^{-1} and 15 g, respectively. The extract was trapped by bubbling the carbon dioxide trough 5 mL of tetrachloroethylene placed in a 10 mL marked flask. After the extraction, the marked flask was filled with tetrachloroethylene.

Tagitinin C contents of *T. diversifolia* aerial parts were quantified by FTIR using a Perkin-Elmer Spectrum GX Fourier Transform Infrared spectrophotometer (Perkin-Elmer Limited, Beaconsfield, England). The absorbance of the very specific C=O stretching vibration (? $_{C=O}$) at 1664.8 cm^{-1} was compared to those of calibration standards to quantify tagitinin C [11].

3. Soxhlet extraction with dichloromethane

200 mg of plant material was extracted with 150 mL of dichloromethane for 2 h in a Soxhlet apparatus. The solution was evaporated to dryness using a rotary evaporator, and the residue was dissolved in 10 mL of tetrachloroethylene.

4. Maceration followed by lixiviation

The extraction and quantification procedures were previously described by Goffin at al. [10].

RESULTS AND DISCUSSION

The optimal conditions were determined using a designed experiment and were met for a pressure of 35.0 MPa and temperature of 67.8°C. The operating procedures were given in a previous work [12].

In the optimized conditions, the addition of polar modifier to the carbon dioxide extraction fluid was examined (Figure 3). Amounts of 1 %(Vol) and 3 %(Vol) of methanol were added but it had no effect on the extraction yield of tagitinin C. This result leads us to think that the extraction is complete at 35.0 MPa and at 68 °C.

The extraction yield and composition of tagitinin C in the extract obtained by the optimized SFE, the Soxhlet extraction with dichloromethane and the maceration followed by lixiviation with ether are presented in Figure 4 and in Figure 5, respectively.

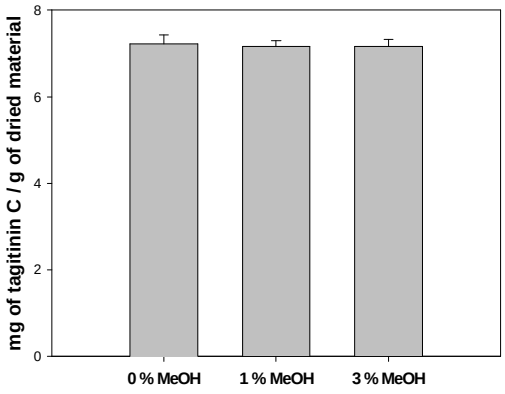

Figure 3: Effect of the amount of methanol on the extraction yield of tagitinin C (n=3). SFE $_{optimized}$ (35.0 MPa, 15g of CO_2, flow rate 1 mL min^{-1}, 68°C).

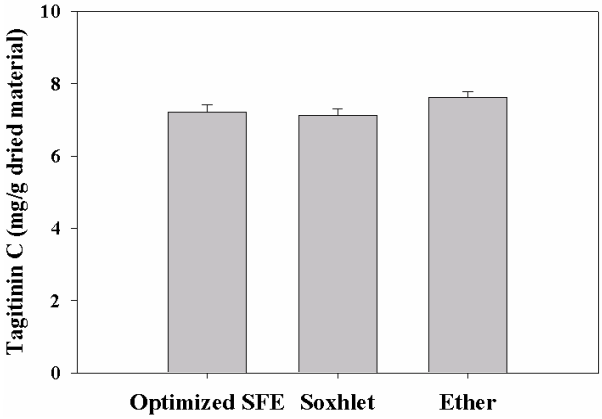

Figure 4: Comparison of different extraction methods: extraction yields of tagitinin C from the dried aerial parts of *Tithonia diversifolia* (n=3).

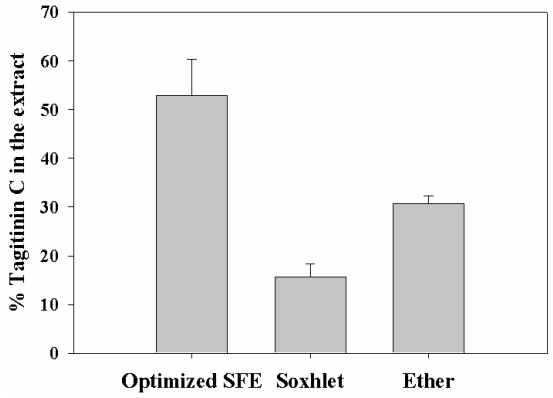

Figure 5: Comparison of different extraction methods: extraction yields of tagitinin C from the dried aerial parts of *Tithonia diversifolia* (n=3).

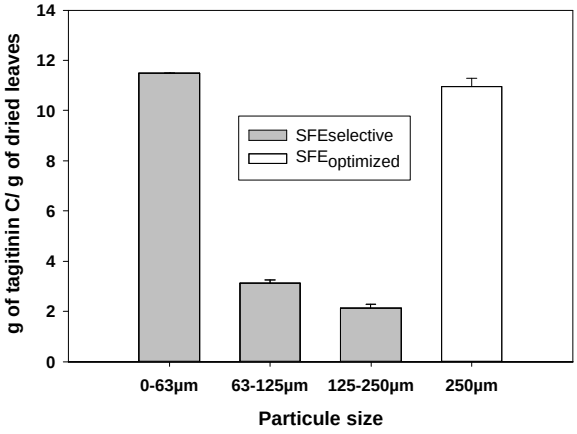

Figure 6: Effect of particle size on extraction yield of tagitinin C from the dried leaves of *T. diversifolia* (n=2). SFE $_{optimized}$ (35.0 MPa, 15g of CO_2, flow rate 1 mL min^{-1}, 68°C). SFE $_{selective}$ (13.7 MPa, 15g of CO_2, Flow rate 1 mL min^{-1}, 40°C).

Figure 4 shows that the yield obtained by the optimized SFE was similar to that by the Soxhlet with dichloromethane and by the maceration followed by lixiviation with ether.

However the supercritical extracts obtained were slightly yellow whereas dichloromethane and ether extracts were dark yellow. Thus, as one can see on the Figure 5, the concentration of tagitinin C in the SFE $_{optimized}$ extract was upper than that of the classical extraction methods. Dichloromethane and ether dissolves pigments from the plant together with the active component.

Under low conditions of pressure (13.7MPa) and temperature (40°C), the particle size (size range 0.02-63, 63-125, 125-250 µm) was investigated to improve the extraction yield of tagitinin C from the leaves of *T. diversifolia* (Figure 6). From the figure 6, it is seen that a diminution of the particle size increases the yield. Furthermore, the range between 0-63µm gives a yield comparable to that of the SFE $_{optimized}$ obtained with leaves sieved to 250 µm size.

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