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Comparison of various extraction methods for identification and determination of volatile metabolites from the brown alga *Dictyopteris membranacea*

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Abstract

Three different methods: hydrodistillation (HD), focused microwave-assisted hydrodistillation (FMAHD) and supercritical fluid extraction (SFE) have been applied, for the first time together, for the extraction of volatile metabolites of the brown alga *Dictyopteris membranacea*. The oils obtained were analyzed by GC–MS (identification and determination of metabolites) and the results were compared. The main chemical classes of compounds identified were C_{11} hydrocarbons for HD method, sesquiterpenes for FMAHD method and sulphur compounds for SFE method.

Keywords: Hydrodistillation; Focused microwave-assisted hydrodistillation; Supercritical fluid extraction; GC–MS; Brown alga; *Dictyopteris membranacea*; Volatile components; Essential oil

1. Introduction

Since Antiquity, natural fragrances hold the attention of man and not surprisingly the first study in the field of natural products chemistry was carried out on aromatic susbtances isolated from terrestrial organisms. In contrast, volatiles of marine origin have been rarely studied although they could be used as a source of original flavouring agents in food and perfume industries. The most characteristic exception is the genus *Dictyopteris* which is one of the few genera of odoriferous brown seaweeds. Many species of this genus have been studied for their essential oil, but only on the basis of hydrodistillation (HD) or “closed-loop-stripping” techniques [1–13] and one time on the one of focused microwave-assisted hydrodistillation (FMAHD) [14].

In this context, the main objective of the present study was to: (i) isolate the volatile fraction obtained from the ether extract of a species of this genus (*D. membranacea*) by conventional HD, FMAHD and supercritical fluid extraction (SFE) with carbon dioxide; (ii) identify and determine (mass percent of compounds) the components of the volatile fraction obtained by each of these three different techniques by GC–MS.

Literature reports that the major constituents identified in the essential oils obtained from algae of the genus *Dictyopteris* were C_{11} hydrocarbons, known as sex pheromones of brown algae [1–3,5,12], sulphur products as 3-hexyl-4,5-dithiacycloheptanone (for *D. plagiogramma* [3], *D. australis* [4] and *D. membranacea* [13]), and sesquiterpene compounds (for *D. prolifera* and *D. undulata*) [6–11].

Concerning the microwave-assisted solvent extraction (MASE), it has been employed for the extraction of different polar and non-polar organic compounds (alkaloids, polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), aromatic hydrocarbons, phenols, phthalic acid esters (PAEs) and pesticides) from various solid matrices (sediment, soil, polymer, plant and animal tissues) [24–29]. The

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Table 1
Retention times and calibration parameters of standard compounds used

Standard compounds, concentration of their stock solution (C in mg/mL) and t_R (min)	Compounds to determine	Calibration curve equation and square of the correlation coefficient (R^2)[a]	RSD (%)	Detection limits (δ) (mg/mL)
1-Undecene $C = 5.2$; $t_R = 6.4$	C_{11} hydrocarbons	$y = 0.958x - 0.010$ $R^2 = 0.999$	0.46	0.022
3-Undecanone $C = 2.9$; $t_R = 12.8$	Two ketones derived from two sulphur compounds and oxygenated C_{11} hydrocarbons	$y = 1.180x - 0.002$ $R^2 = 0.999$	0.46	0.004
α-Copaene $C = 3.2$; $t_R = 15.8$	Sesquiterpene hydrocarbons	$y = 1.081x - 0.003$ $R^2 = 0.999$	0.16	0.001
Cedrol $C = 2.7$; $t_R = 24.5$	Oxygenated sesquiterpenes	$y = 1.161x + 0.004$ $R^2 = 0.999$	0.03	0.025
Tetradecanoic acid $C = 2.5$; $t_R = 30.1$	Tetradecanoic acid, alkanes	$y = 1.793x + 0.024$ $R^2 = 0.999$	0.22	0.150
1,3-Di(2-thienyl)-1-oxoprop-2-ene $C = 3.3$; $t_R = 39.7$	Hexadecanoic acid and derivatives	$y = 2.280x + 0.009$ $R^2 = 0.999$	0.62	0.140
Hexadecanoic acid $C = 3.0$; $t_R = 36.7$	3-Hexyl-4,5-dithiacycloheptanone	$y = 1.583x + 0.025$ $R^2 = 0.999$	0.31	0.172

[a] $y = C_s/C_{is}$ and $x = A_s/A_{is}$ where C_s, A_s = concentration and peak area of standard, C_{is}, A_{is} = concentration and peak area of internal standard.

usefulness of microwave irradiation as a heating source for the assistance of wet ashing techniques was demonstrated in 1975 [30]. It has mainly assisted digestion steps [31–33]; nevertheless, it has also been successfully used to help leaching steps. The first in this field was Ganzler [24,25] who used a domestic microwaves oven in order to demonstrate a higher extraction of polar compounds by this leaching, compared with the Soxhlet method. Noticeable contributions to expand the use of microwaves as an alternative to conventional methods have been reported by Paré et al. [34] and Lopez-Avila et al. [35–37], using focused and multimode microwaves. It must be pointed out that the FMAHD of volatile oils [14,38] seems to be an efficient technique, particularly with the great reduction of extraction time.

For the SFE, it has gained increasing attention over the traditional techniques like steam distillation and liquid solvent extraction. The use of a non-toxic and volatile solvent, such as supercritical CO_2, protects extracts from thermal degradation and solvent contamination [15], leading to high quality products [16,17]. Despite the number of SFE experimental data reported for many natural species, the modelling and simulation of this process remains a major issue of research activity, justifying the need of more reliable experimental data. So far, the extraction process has been described by different models: empirical models [18,19], models based on heat transfer analogy [20–22], by assuming that the vegetable particle is a single sphere cooled in an uniform fluid medium, and finally mass balance models [23].

2. Experimental

2.1. Plant material and chemicals

The plant material was collected off the Mediterranean coast of Algeria (La Corne d'Or, Tipaza) in September 2002. This sample belongs to: Phaeophyceae Class, Dictyotales order, Dictyotaceae family, *Dictyopteris* genus and *membranacea* (Stackhouse) Batters species [39]. A voucher specimen of this species was deposited in the herbarium of Dr. M. Pellegrini, Laboratoire de Biologie Marine Fondamentale et Appliquée, Université de la Méditerranée, Marseille, France. *D. membranacea* was air-dried under shade without any other treatment.

Non-stabilized diethyl ether, dichloromethane and ethyl acetate of analytical grade were purchased from Carlo Erba (Val de Reuil, France). Pure standards used for calibration curves (Table 1) were from Sigma–Aldrich (Saint-Quentin Fallavier, France).

2.2. Extractive techniques

2.2.1. Solvent extraction

The extraction was performed in a 2.5 L-round flask with 300 g of plant material and 2 L of diethyl ether (batch extraction). The solvent was removed by vacuum distillation leading to 3.5 g of a crude extract (concrete), that is to say a yield of 1.2%.

2.2.2. Preparation of the volatile fractions

The volatile fractions studied in the present work were obtained from the crude extract with three different techniques described below.

2.2.3. Hydrodistillation (HD)

It was performed with a modified Dean-Stark system. The crude extract (266 mg) was crossed by a stream of steam over 1 h with 300 mL of water. Liquid–liquid extraction with diethyl ether was used to obtain the essential oil (yield: 12.9% of the crude extract).