SUBCRITICAL WATER EXTRACTION OF ANTIOXIDANT COMPOUNDS FROM *Phagnalon sordidum* (L.).

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ABSTRACT

In the present study, Subcritical Water Extraction (SWE) was investigated as green approach for the extraction of antioxidant from Corsican *Phagnalon sordidum*, a perennial weed widespread in the Mediterranean region. The SWE process carried out at four temperatures (100°C, 125°C, 150°C, 175°C) and at the same pressure (6.10^6 Pa), was evaluated in terms of quantitative and qualitative phenolic composition using HPLC-DAD and antioxidant activity using the DPPH and ORAC (Oxygen Radical Absorbance Capacity) assays.

The main concentration of total phenolic compounds (150 ± 7 mg Eq. Gallic Acid/g Dry Matter) was obtained by SWE processed at 125°C. This result was 1.5 times higher than that obtained by a conventional extraction using ethanol maceration (100 ± 6 mg Eq. Gallic Acid/g Dry Matter). The qualitative phenolic composition of the extracts was also studied: 3-O-Caffeoylquinic acid and derivatives of caffeoylquinic acids were identified and their concentrations varied with the extraction temperatures. The extract obtained at 125°C exhibited significant antiradical activities, with IC50 of 22.9 ± 1.9 µg/mL and an ORAC value of 5293 ± 294 (µmol Trolox/g Dry Matter).

INTRODUCTION

The search for new antioxidant compounds is an important challenge for pharmaceutical, cosmetic and food industries. Environment strategy development for the extraction of these compounds is advised. In recent years, a number of new solvent-free extractions techniques which preserve environment and human safety were developed [1]. Among them, Subcritical Water Extraction (SWE) is a green extraction that offers many advantages. It is a technique based on the use of water, at temperature between 100°C and 374°C and pressure hight enough to maintain the liquid state [2]. Under this condition,

dielectric constant of water is the same as that organic solvents dielectric constant (ethanol, methanol) at ambient temperature, indicating that water can be used for extracting hydrophobic compounds from natural plants. SWE was studied these recent years and many of studies reported comparable values or better recoveries yields than using organic solvent extraction [3]. This technique seems to be well adapted for polyphenols extraction [1,4].

In this study, SWE was developed as a green approach for a perennial Mediterranean plant: *Phagnalon sordidum*. The genus *Phagnalon* belongs to the Asteraceae family and includes about 36 species distributed worldwide. In the medicinal folk traditions of the Balearic Islands, *P. sordidum* is used alone or mixed with *Lippia citriodora* and/or *Malva sylvestris* in order to cure renal calculosis [5]. From a pharmacology point of view, the genus *Phagnalon* has been the subject of several scientific reports [6–9].

For our best knowledge, there is no report on biological activities and polyphenols characterization of *P. sordidum*. In this study, we developed SWE applied at different temperatures for polyphenols extraction of *P. sordidum*. In a second step, polyphenols were characterized and quantified, and the antioxidant activity of the extracts was studied.

MATERIALS AND METHODS

The aerial parts of *P. sordidum* (L.) were collected in June 2013 (Corsica, France) and stored in the dark. Subcritical Water Extractions were performed from aerial parts of *P. sordidum*, 3.9 g were inserted in the extractor cell. Four water temperatures were tested: 100°C, 125°C, 150°C and 175°C. The extraction was carried out during 1 h in order to obtain a volume of extract fixed at 400 mL. Total phenolic content was determined according to the Folin-Ciocalteu method using gallic acid as the standard and expressed in Gallic Acid Equivalent (GAE). Extracts were analyzed using HPLC-DAD and the compounds were identified by comparing their retention times and UV-VIS spectra with authentic standards.

The antioxidant capacity was determined using the DPPH and ORAC methods [10].

RESULTS

Subcritical water extraction process was applied to aerial parts of *P. sordidum* at different temperatures and was compared to hydro-alcoholic maceration (Table 1).

Table 1: Extraction from aerial parts of *P. sordidum*, yield and total phenol content

Method of extraction	Extraction temperature (°C)	Yield (%) ± SD*	Total Phenol (mg GAE/g) ± SD* of Dry Matter (DM)
Maceration	25	15.7 ± 1.7	100 ± 6
Subcritical water	100	12.3 ± 0.9	133 ± 5
	125	20.5 ± 0.7	150 ± 7
	150	29.9 ± 3.1	114 ± 12
	175	37.2 ± 4.2	108 ± 4

*Data expressed as mean ± standard deviation (SD) of three replicates.

The optimal temperature of extraction using SWE was obtained at 175°C, with an extraction yield > 2 times higher than maceration (37.2 ± 4.2 % and 15.7 ± 1.7 %, respectively).

However, Table 1 shows that the total phenolic content in the extracts was higher at 125°C compared to 175°C (150 ± 7 vs 108 ± 4 mg GAE/g DM), which suggests that some additional compounds are extracted at high temperatures.

HPLC-DAD analysis of the extracts showed the presence of 5-O-Caffeoylquinic acid (**1**), chlorogenic acid (3-O-Caffeoylquinic acid) (**2**), 4-O-Caffeoylquinic acid (**3**), p-coumaric acid (**4**), 3,4-Dicaffeoylquinic acid (**5**) and 3,5-Dicaffeoylquinic acid (**6**) (Figure 1). Total caffeoylquinic acids quantification (Table 2) indicates that the higher concentration was obtained at 125°C (6.9 %).

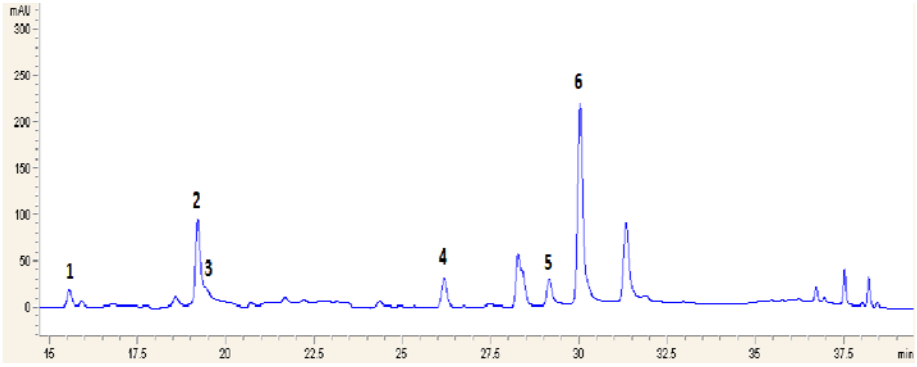

Figure 1: HPLC chromatogram of the *P. sordidum* subcritical water extract acquired at λ = 306 nm.

Table 2: Effect of temperature on total contents of caffeoylquinic acids.

Temperature (°C)	Total caffeoylquinic acids (% DM)
100	5.7
125	6.9
150	5.7
175	3.3

The effect of the temperature on the caffeoylquinic acids content in the extract was shown in Figure 2. Except for 3,4-dicaffeoylquinic acid, highest concentrations were obtained at 125°C and an important decrease was observed at higher temperatures (>150°C). Thus, the 125°C temperature was selected as optimum temperature.

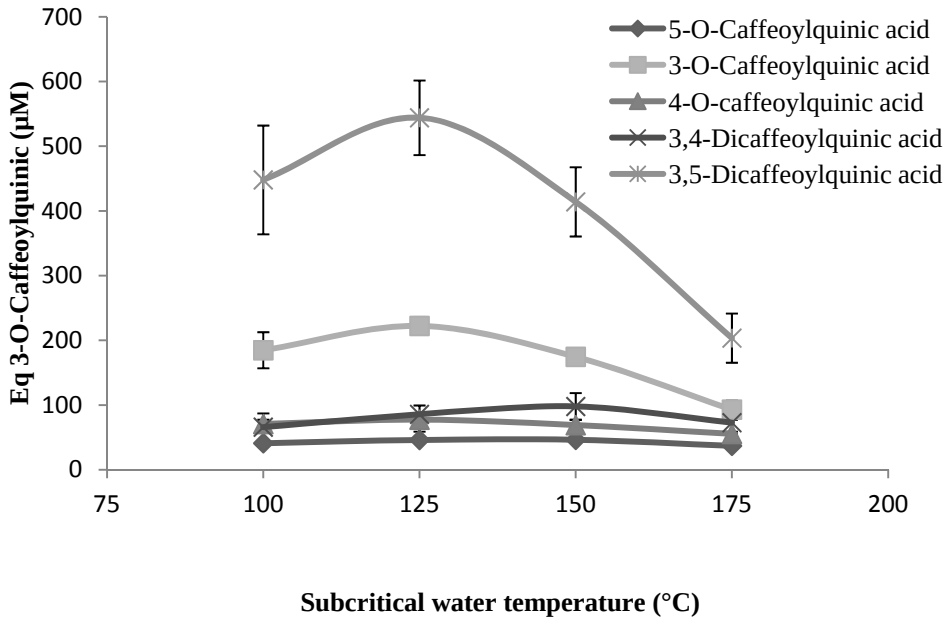

Figure 2: Effect of temperature on the caffeoylquinic acids contents of the extracts.

Table 3 shows the results of the free radical scavenging activity of the *P. sordidum* extract using the DPPH and ORAC assays:

Table 3: Free-radical scavenging activity of the SWE extract.

	DPPH IC_{50} (µg/ml)	ORAC (µmol Trolox/g DM)
125°C SWE	22.9 ± 1.9	5293 ± 294
Trolox	4.2 ± 0.1	

Relative to literature data, *P. sordidum* extract obtained by SWE exhibited significant antioxidant activity.

CONCLUSION

Subcritical Water extracts from aerial parts of *P. sordidum* are interesting since they displayed large amounts of 3,5-dicaffeoylquinic acid and endowed efficient antioxidant activity. The results of the present study show that Subcritical Water Extraction is a good alternative to organic solvents for polyphenols extraction. Beside, SWE offers many advantages over traditional extraction, namely: shorter extraction times (1 hour against 10 h

for maceration); solvent-free; the possibility of manipulating the chemical composition of the extract according to the temperature; non toxic for human.

These results are promising and demonstrate the practical feasibility of SWE to substitute the traditional time-consuming techniques for efficient extraction of antioxidant actives to provide cosmetics companies.

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