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Antioxidant activity of hydro distillation water residues from *Rosmarinus officinalis* L. leaves determined by DPPH assaysAlexander Wollinger^a, Élodie Perrin^a, Jamal Chahboun^b, Valérie Jeannot^b,
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ARTICLE INFO

Article history:

Received 3 September 2015

Accepted 3 December 2015

Available online xxxx

Keywords:

Antioxidants

Rosemary

Essential oil

Steam distillation

Hydro distillation

DPPH

Antioxidant activity

ABSTRACT

Rosemary (*Rosmarinus officinalis* L.) is a perennial herb with an intensive aromatic flavor. Its most important chemical constituents are essential oils (e.g., 1,8-cineole and camphor) and antioxidants (e.g., carnosic acid and rosmarinic acid). The common methods to extract the essential oil of rosemary are steam or hydro distillation. The aim of this work is to investigate the residual antioxidants after hydro distillation, especially rosmarinic acid and carnosic acid. For this purpose, the hydro distillation water residues were analyzed by HPLC-UV. Moreover, the influence of the extraction duration on the concentration of the antioxidants was investigated. Also, the residual amount of these compounds in the leaves was examined. The total antioxidant activity of the extracts and of the pure compounds was determined by DPPH assays. It is shown that after 2.5 h of hydro distillation the amount of rosmarinic acid and the antioxidant activity in the water residue reaches a maximum value. In addition, the yield and the quality of the essential oil were investigated to draw a comparison between steam and hydro distillations of Moroccan rosemary leaves.

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R É S U M É

Mots-clés:

Antioxydants

Romarin

Huile essentielle

Distillation par entraînement à la vapeur

Hydrodistillation

DPPH

Activité anti-oxydante

Le romarin (*Rosmarinus officinalis* L.) est une plante aromatique pérenne. Ses composants chimiques principaux sont les huiles essentielles (par exemple de 1,8-cineol, de camphre) et les antioxydants (par exemple l'acide carnosique, l'acide rosmarinique). Les méthodes traditionnelles pour extraire l'huile essentielle de romarin sont la distillation par entraînement à la vapeur et l'hydrodistillation. L'objectif de ce travail est l'étude des antioxydants résiduels après l'hydrodistillation, en particulier de l'acide rosmarinique et carnosique. Pour atteindre cet objectif, l'eau résiduelle de l'hydrodistillation a été analysée par HPLC-UV. De plus, l'influence de la durée d'extraction sur la concentration des antioxydants a été étudiée. La quantité résiduelle de ces composants dans les feuilles a également été examinée. L'activité antioxydant totale de ces extraits et celle des composants purs a été déterminée en utilisant le DPPH comme molécule test. Il a été montré que la quantité d'acide rosmarinique et l'activité antioxydant de l'eau résiduelle atteignent une valeur maximale après 2,5 h d'hydrodistillation. Le rendement et la qualité de l'huile essentielle ont également été étudiés pour comparer la distillation par entraînement à la vapeur et l'hydrodistillation des feuilles de romarin marocain.

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<http://dx.doi.org/10.1016/j.crci.2015.12.014>

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1. Introduction

Rosemary (*Rosmarinus officinalis* L.) is a perennial shrub, which is originated in the Mediterranean area. The plant is also cultivated in Spain, Morocco, Tunisia, and the south-east of Europe. Leaves of rosemary have an intense aromatic flavor and bitter, slightly spicy taste. Rosemary is widely used for seasoning and flavoring foods, as a preservative agent and an antioxidant. Also pharmaceutical applications are known [1].

The essential oil from rosemary is commonly gained by hydro (HD) or steam distillation (SD) with a maximum extraction yield of 1.0–2.5%. The colorless or slightly yellow oil contains 1,8-cineole (15–30%), camphor (10–25%), α -pinene (10–25%), and borneol (3–20%). The chemical structures of these substances are presented in Fig. 1. Other compounds are bornyl acetate (1–5%), camphene (5–10%), α - β -terpineol, myrcene, limonene, and caryophyllene. Essential oils from Spain or Tunisia can additionally contain a relatively high amount of verbenone. The ratio of these terpenes varies depending on the origin and chemo type of the rosemary plant [1–3]. The essential oil is located in glandular trichomes on the surface of the rosemary leaves [4]. Rosemary oil is used as an antibacterial, antifungal, and anticancer agent [5]. Hydro and steam distillations are easy methods to extract the essential oil from rosemary leaves. For hydro distillation, rosemary leaves and water are put together into a flask. The suspension is heated until boiling. This procedure is in contrast to steam distillation, where the steam is generated in a separate flask and guided through the plant material. The steam takes the essential oil along and the water/oil mixture and is condensed. A two phase system with water and the essential oil is produced, where the oil can be decanted and recovered [6]. The distilled and condensed water phase is called hydrosol. If this hydrosol is recycled and taken to carry out another steam or hydro distillation the process is

called cohobation [7]. Hydro and steam distillations merely work because of the coexistence of two immiscible liquids (water and essential oil). The vapor pressure of the system is equal to the sum of the vapor pressures of the pure compounds. The boiling point of the mixture is lower than the boiling points of water and the essential oil. Thus, the essential oil can be extracted without reaching the boiling point of the single compounds. A limitation of this method is that low volatile substances can only be recovered in small quantities [2,8]. Alternative methods to extract the essential oil from rosemary are supercritical carbon dioxide extraction [5,9] and subcritical water extraction [10].

Antioxidants (AO) are compounds which can inhibit or retard the oxidation of lipids and other biomolecules. They prohibit the start of an oxidizing chain reaction by radicals or quench the propagation. These reactions can cause functional damage to the human body, like cancer or cardiovascular diseases. Antioxidants can prevent this process due to their redox properties like reductive behavior, the donation of hydrogen or quenching of singlet oxygen [11,12]. Rosemary is one of the major resources for natural antioxidants. The most important compounds are the phenolic diterpene carnosic acid (Cac) and the phenolic rosmarinic acid (Rac). Carnosol (CA) and rosmanol are formed by oxidative degradation of carnosic acid and are not contained initially in the leaves. Thus, these compounds are artifacts of the extraction process. The chemical structures of these antioxidants are shown in Fig. 1 [3,13]. The content of these antioxidants in the leaves varies in a large range due to seasonal variations, environmental influences, species, and growing origin. Also large fluctuations in the individuals of the same population have been reported. In the literature the content of carnosic acid varies from 4 to 30 mg per 1 g of rosemary. The mass concentration of rosmarinic acid in the leaves is in the range between 2 and 25 mg/g [14–16]. However, these compounds do not only show antioxidant activity.

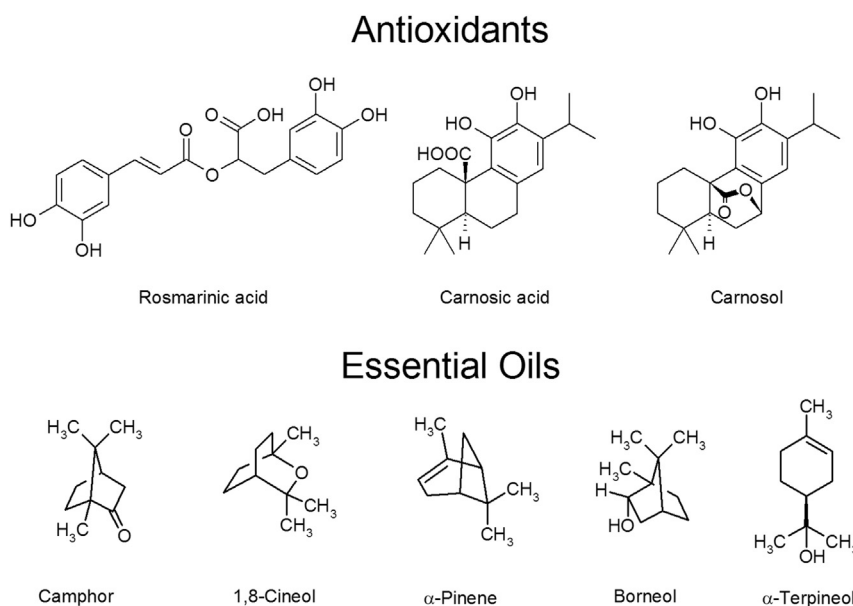


Fig. 1. Chemical structures of the main compounds present in rosemary leaves, subdivided into antioxidants (rosmarinic acid, carnosic acid, and carnosol) and essential oils (camphor, 1,8-cineol, α -pinene, borneol, and α -terpineol).

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