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^{1}H NMR based metabolic profiling of eleven Algerian aromatic plants and evaluation of their antioxidant and cytotoxic properties

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ABSTRACT

Eleven Algerian medicinal and aromatic plants (*Aloysia triphylla*, *Apium graveolens*, *Coriandrum sativum*, *Laurus nobilis*, *Lavandula officinalis*, *Marrubium vulgare*, *Mentha spicata*, *Inula viscosa*, *Petroselinum crispum*, *Salvia officinalis*, and *Thymus vulgaris*) were selected and their hydroalcoholic extracts were screened for their antiradical and antioxidant properties in cell-free systems. In order to identify the main metabolites constituting the extracts, ^{1}H NMR-based metabolic profiling was applied. Data obtained emphasized the antiradical properties of *T. vulgaris*, *M. spicata* and *L. nobilis* extracts (RACI 1.37, 0.97 and 0.93, respectively), whereas parsley was the less active as antioxidant (RACI −1.26). When the cytotoxic effects of low and antioxidant doses of each extract were evaluated towards SK-N-BE(2)C neuronal and HepG2 hepatic cell lines, it was observed that all the extracts weakly affected the metabolic redox activity of the tested cell lines. Overall, data strongly plead in favor of the use of these plants as potential food additives in replacement of synthetic compounds.

1. Introduction

Plants used primarily for their medicinal or aromatic properties in pharmacy or perfumery are defined as medicinal and aromatic plants (MAPs). Many plants defined as MAPs are also used for cosmetic purposes, so that the definition medicinal, aromatic and cosmetic (MAC) plants would better describe such plants (Lubbe & Verpoorte, 2011). In terms of production of industrial products/fine chemicals from plants, some plants defined as MAPs and MACs can also be used in the production of dyes, colorants and crop protection products. Health products, nutraceuticals or dietary supplements based on medicinal and aromatic plants have also gained popularity. The beneficial properties of these plants seemed to be ascribed to their content in phytochemicals (Dias, Barros, Sousa, & Ferreira, 2012), and in particular to their richness in phenolic compounds (Guimarães et al., 2013).

Polyphenols are bioactive secondary metabolites, commonly found in both edible and inedible plants (Wojdyło, Oszmiański, & Czemerys, 2007; da Silva Port's, Chisté, Godoy, & Prado, 2013; Kaiser, Kammerer, & Carle, 2013), which, nowadays, attract special attention due to their health-promoting characteristics (Sumbul, Ahmad, & Mohd, 2011). Phenolic compounds in plants are closely associated with their antioxidant activity, which is mainly due to their redox properties and their capacity to counteract the over-production of reactive oxygen species (Sharma, Bhushan Jha, Shanker Dubey, & Pessarakli, 2012). These natural products, with considerable diversity in their structure, contribute to flavor, color and sensory properties of plants. Cells respond to polyphenols mainly through direct interactions with receptors or enzymes involved in signal transduction, which may result in modification of the redox status of the cell and may trigger a series of redox-dependent reactions (Scalbert, Johnson, & Saltmarsh, 2005). Both antioxidant and prooxidant effects of polyphenols have been described, with contrasting effects on cell physiologic processes. As antioxidants, polyphenols may improve cell survival; as prooxidants, they may induce apoptosis and prevent tumor growth. However, the biological effects of polyphenols may extend well beyond the modulation of oxidative stress (Scalbert et al., 2005). The high antioxidant power of herbal extract is of particular interest to food industry. In fact, oxidative deterioration of food products during processing and storages produces off-flavor, which affects their marketability, and different oxidized metabolites, which seem to be involved in some disease conditions (Hossain, Brunton, Barry-Ryan, Martin-Diana, & Wilkinson, 2008). As the use of synthetic antioxidants, such as butyl hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butylated hydroquinone (TBHQ) has been strongly regulated due to their toxic effects, medicinal and aromatic plants are thought to

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be sources of natural antioxidants, which could potentially replace the synthetic antioxidants offering additional health benefits.

In Algeria, collection of medicinal and aromatic plants to extract, after distillation, essential oils for the manufacture of cosmetics, pharmaceuticals as well as flavors for food products, is a virgin field (Reguieg, 2011) but little is known about Algerian medicinal plants phenolic content and their potential as source of active antioxidant ingredients (Djeridane et al., 2006; Atmani et al., 2009).

In this context, eleven Algerian medicinal and aromatic plants (*Aloysia triphylla*, *Apium graveolens*, *Coriandrum sativum*, *Laurus nobilis*, *Lavandula officinalis*, *Marrubium vulgare*, *Mentha spicata*, *Inula viscosa*, *Petroselinum crispum*, *Salvia officinalis*, and *Thymus vulgaris*) were selected and their hydroalcoholic extracts were analyzed through ^{1}H NMR spectroscopy, an effective technique for both metabolite fingerprinting and metabolite profiling applications in samples of plant origin. All the extracts were also screened for their antiradical and antioxidant properties in cell-free systems. The cytotoxic effects of low doses of each extract were evaluated towards neuronal and hepatic cell lines. The choice of our investigated plants is based on two criteria: first, in this domain there is no study in Algeria that deals with these plants, and the second criterion is that these plants have ethnopharmacological data indicating their traditional utilization in the treatment of different diseases and disorders (Paula de Oliveira et al., 2011). In addition, some of them have been proved to be efficient in the treatment of various cancerous lesions (Xavier, Lima, Fernandes-Ferreira, & Pereira-Wilson, 2009; Aissaoui, Zizi, Israili, & Lyoussi, 2011; Sertel, Eichhorn, Plinkert, & Efferth, 2011; Talib, Zarga, & Mahasneh, 2012) and as potential neuroprotectants (Vora, Patil, & Pillai, 2009; Kelsey, Wilkins, & Linseman, 2010; Pacifico et al., 2013; Pacifico et al., 2014). Due to their traditional utilization and active components, these plants are also considered to be efficient for the treatment of free radical-related disorders.

2. Materials and methods

2.1. Reagents and chemicals

All of the solvents and reagents used for assessing antioxidant screening were purchased from Sigma-Aldrich Chemie (Buchs, Switzerland) except ABTS, which was from Roche Diagnostics (Roche Diagnostics, Mannheim, Germany). Cell culture medium and reagents for cytotoxicity testing were purchased from Invitrogen (Paisley, UK); MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] was from Sigma-Aldrich Chemie. Deuterated solvents and internal standard for NMR-based metabolic profiling analyses were purchased from Sigma-Aldrich Chemie.

2.2. Plant collection and fractionation

Leaves of the eleven selected plants were collected in October 2012 at Souk El Tenine Nature Reserve (Bejaia, Algeria) and identified by Dr. Ourabah of the Faculté des Sciences Université de Constantine (Algeria). Three replicate samples (10.0 g each) of leaves of each selected plant, cleaned, were dried in a ventilated thermostat at 40 °C for 72 h and ground to powder using a porcelain mortar and pestle, until fine and homogeneous particles were obtained. Aliquots of dried leaves (1.0 g) underwent ultrasound-assisted extraction (Dr. Hielsher UP 200S, Germany) using an hydro-alcoholic solution (10.0 mL; H_2O:MeOH; 1:1, v:v) as extracting solvent. Four sonication cycles were performed (30 min each) in order to achieve the maximum recovery of the leaf metabolite content. At the end of each sonication cycle, samples were centrifuged at 2044 $\times g$ for 10 min in a Beckman GS-15R centrifuge (Beckman Coulter, Milano, Italy) fitted with rotor S4180. Obtained supernatants were dried under vacuum by a rotary evaporator (Heidolph Hei-VAP Advantage, Germany) to yield crude extracts, which were stored at −20 °C until use.

2.3. NMR-based metabolic profiling

Algerian aromatic plants hydroalcoholic extracts (40.0 mg each) were dissolved in 1.0 mL of a solvent system made up of K_2HPO_4 buffer (pH 6.0, 90 mM) in D_2O (containing the internal standard) and methanol-d_4 (1:1 v/v). Trimethylsilylpropionic-2,2,3,3-d_4 acid sodium salt (TSP-d_4, 0.1%, w/v) was used as internal standard. NMR spectra were recorded at 25 °C on a Varian Mercury Plus 300 Fourier transform NMR at 300.03 MHz for ^{1}H and at 75.45 MHz for ^{13}C. Spectra were calibrated by setting the TSP peak at 0.00 ppm. Data acquisition parameters, for the ^{1}H NMR spectrum, were as follows: 0.16 Hz/point, acquisition time, number of scans (NS) = 256, relaxation delay (RD) = 1.5 s, 90 pulse width, number of data points (NP) = 4096, spectral width = 3065 Hz. A presaturation sequence was used to suppress the residual H_2O signal. Line broadening of 0.3 Hz and zero-filling to 64 K were applied prior to Fourier transform. FIDs were Fourier-transformed, and the resulting spectra were manually phased and baseline-corrected using an ^{1}H NMR processor (MestreNova 8.0). Plant extract composition has been defined by comparing NMR data with an in-house library, with databases (Cui et al., 2008) and with some literature data (Lubbe, Gude, Verpoorte, & Choi, 2013; Verpoorte, Choi, & Kim, 2007; Wolfender, Rudaz, Choi, & Kim, 2013). For some metabolites, the support of 2D-NMR techniques was needed.

2.4. Determination of total phenol content

Total phenol amount of both crude extracts was determined according to the Folin–Ciocalteau procedure (Pacifico et al., 2012). Analyzed samples (1.0 mg/mL in DMSO) were mixed with 0.500 mL of Folin–Ciocalteau reagent (FCR) and 4.0 mL of Na_2CO_3 (7.5% w/v). After stirring reaction mixture at room temperature for 3 h, the absorbance was read at 765 nm using a Shimadzu UV-1700 spectrophotometer (Shimadzu, Salerno, Italy). The content of total phenols of the samples was expressed as milligram gallic acid equivalents (GAEs) per 100 g of fresh material.

2.5. Determination of total flavonoid content

The flavonoid content of methanolic extracts was measured using a colorimetric assay as reported in Piccolella et al. (2008). A known volume (0.5 mL) of the extract or standard solution of catechin was added to a 10 mL volumetric flask. Distilled water was added to make a volume of 5 mL. At 0 time, 0.3 mL of $NaNO_2$ (5%, w/v) was added to the flask. After 5 min, 0.6 mL of $AlCl_3$ (10%, w/v) was added, and after 6 min, 2 mL of NaOH (1.0 M) was added to the mixture followed by the addition of 2.1 mL of distilled water. Absorbance was read at 510 nm against the blank (water), and flavonoid content was expressed as milligrams of catechin equivalents (CAT) per 100 g of fresh material.

2.6. Antioxidant activity assessment

Because multiple reaction characteristics and mechanisms as well as different phase localizations are usually involved, no single assay can accurately reflect all types of radical sources or antioxidants in a mixed or complex system. Thus, five different in vitro anti-radical assays were employed. Hydroalcoholic extracts from investigated Algerian aromatic plants, previously dissolved in DMSO as stock solutions of 12.5 mg/mL, were evaluated at different concentration levels (DMSO final concentration was equal to 0.1% (v/v)). Tests were carried out performing three replicate measurements for three samples (n = 3) of each extract (in total, 3 × 3 measurements). Recorded activities were compared to a blank. Results are the mean ± SD values. Student *t*-test was applied in order to determine statistical significance (significance level was set at $P < 0.05$).

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