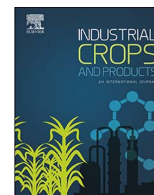
Industrial Crops & Products 108 (2017) 616–628

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop



Phytochemical analysis and evaluation of the antioxidant and antimicrobial properties of selected herbs cultivated in Greece

Eirini Anastasaki[a], Georgia Zoumpopoulou[b], Konstantina Astraka[a], Eleftheria Kampoli[b], Georgia Skoumpi[a], Konstantinos Papadimitriou[b], Effie Tsakalidou[b], Moschos Polissiou[a,*]

[a] Laboratory of Chemistry, Department of Food Science and Human Nutrition, School of Food, Biotechnology and Development, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece
[b] Laboratory of Dairy Research, Department of Food Science and Human Nutrition, School of Food, Biotechnology and Development, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece

ARTICLE INFO

Keywords:
Herbal extracts
Phytochemical analysis
Antioxidant activity
Antimicrobial activity
Anti-biofilm activity

ABSTRACT

In this study, we investigated the bioactivity of extracts of Lamiaceae and Asteraceae family plants after sequential extraction with petroleum ether (PE), diethyl ether (DE) and methanol (ME). The phytochemical analysis showed that the major components identified in the PE extracts were volatile compounds. DE extracts were rich in aglycone flavonoids and terpene derivatives and in ME extracts phenolic acids and flavonoids glycosides, were found. ME extracts presented stronger scavenging activity towards 2,2-diphenyl-1-picrylhydrazyl (DPPH·) and 2,2′-azino-bis (3- ethylbenzthiazoline)-6-sulfonic acid (ABTS$\cdot^{+}$) free radicals than PE and DE extracts. Fourier-Transform Infrared Spectroscopy (FTIR) combined with principal component analysis (PCA) revealed that exposure of the target cells of oral pathogens to the ME extracts caused major alterations of cellular components. Lemon balm and chamomile ME extracts reduced biofilm formation of *Streptococcus mutans* and *Streptococcus sobrinus* strains. This study highlights that ME extracts may be good candidates as plant-derived antioxidants and antimicrobial agents.

1. Introduction

Since ancient times and throughout the world, medicinal and aromatic plants have been used both for their healing properties and their ability to provide a distinctive flavor and aroma to food. Indeed, natural products have been a rich and promising pool of novel biologically active compounds. The food and pharmaceutical industries use chemical additives and antiseptics in several processes to extend the shelf life of foods and prevent or treat microbial infections, respectively. Given the adverse effects reported for synthetic chemical compounds and the demand of consumers for natural food additives/preservatives (Bearth et al., 2014), researchers have focused on the discovery of naturally occurring plant-derived antioxidants and antimicrobials (Embuscado, 2015; Hintz et al., 2015).

Plant compounds with these bioactivities belong mostly to flavonoids, phenolic acids, phenolic diterpenes (Pizzale et al., 2002) and terpenoids (Daferera et al., 2000). Antioxidants act directly *via* free radical scavenging mechanisms and/or indirectly by preventing the reactive oxidants from being formed in the first place (Huang et al., 2005). Antimicrobial properties of plant-derived compounds are linked to the structural and/or functional damage of the target cells (Savoia, 2012).

During the last decade, the antioxidant and antimicrobial activities of both essential oils and extracts of Asreraceae and Lamiaceae family have been extensively studied. Their properties regarding the elimination of pathogenic microorganisms, as well as the reduction of lipid oxidation, have been the basis of their application in active food packaging (Ribeiro-Santos et al., 2017). Extracts rather than dried leaves or essential oils are preferred for use in food, cosmetic and pharmaceutical industries, due to limitations arising from the strong smell or taste (Ferrazzano et al., 2009) or their potential toxicity in high doses (Skotti et al., 2014; Tajkarimi et al., 2010). De-odorized extracts may be the solution. Sequential extraction with solvents of increased polarity can result in de-odorized extracts enriched with antioxidant and antimicrobial constituents (Garmus et al., 2015). Up to now, scarce literature exists for the above process in the aforementioned families and the resulting bioactivities (Kouri et al., 2007).

Furthermore, among antimicrobial activities of plant extracts, identification of effective natural agents active in biofilms is a topic of significant importance since, once firmly established, a biofilm can be

* Corresponding author.
E-mail address: mopol@aua.gr (M. Polissiou).

http://dx.doi.org/10.1016/j.indcrop.2017.06.066
Received 18 January 2017; Received in revised form 24 June 2017; Accepted 30 June 2017
0926-6690/ © 2017 Elsevier B.V. All rights reserved.

very difficult to eradicate (Olsen, 2015). Thus, it is worth investigating natural compounds for developing innovative strategies to counteract biofilm resistance properties to antimicrobials (Borges et al., 2016). Due to major implications of certain *Streptococcus* species in oral health, several studies have been conducted to evaluate the efficacy of phytochemicals in the reduction of their biofilm and plaque formation (Abachi et al., 2016). In this context, the control of dental biofilms by natural compounds has become a major objective of scientific research (Kouidhi et al., 2015). Indeed, essential oils and plant derived compounds have been tested against oral pathogens like *Streptococcus mutans* (Ciandrini et al., 2014; Rasooli et al., 2009). However, limited studies exist on Lamiaceae and Asteraceae plant extracts against biofilms in general (Bazargani and Rohloff, 2016; Sandasi et al., 2011).

The present study aims to determine the phytochemical profile of extracts derived from five herbs widely used in the Mediterranean diet, namely chamomile (*Matricaria recutita* L., Astreaceae), dittany (*Origanum dictamnus* L., Lamiaceae), lemon balm (*Melissa officinalis* L., Lamiaceae), rosemary (*Rosmarinus officinalis* L., Lamiaceae) and sage (*Salvia officinalis* L., Lamiaceae) after a sequential extraction with solvents of increased polarity, to evaluate both antioxidant and antimicrobial activity of these extracts against pathogenic and food-spoilage bacteria and finally to examine the anti-biofilm activity of lemon balm and chamomile methanolic extracts on selected *Streptococcus* species.

2. Materials and methods

2.1. Reagents and standards

Folin-Ciocalteau phenol reagent, potassium persulfate ($K_2S_2O_8$), sodium carbonate (Na_2CO_3), absolute ethanol and petroleum ether (bp 40–60°) were supplied by Merck KGaA (Darmstadt, Germany). Diethyl ether (pesticide pure) and methanol were obtained from Carlo Erba (Val de Reuil, France), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Standard compounds of α-bisabolol, borneol, carnosic acid, carvacrol, *β*-caryophyllene, citral, citronellal, *p*-cymene, eriodictyol, eucalyptol, ferulic acid, gallic acid, geranyl acetate, limonene, linalool, narigenin, α-pinene, rosmarinic acid, γ-terpinene, thymol and thymoquinone were purchased from Sigma-Aldrich (St. Louis, MO, USA). 1,4-Dioxaspiro [4.4]non-6-ene (Santa Cruz Biotechnology, Inc., Texas, USA). Apigenin, chlorogenic acid (3-*O*-Caffeolyquinic acid), cynarin (1,5-*O*-Dicaffeoylquinic acid), genkwanin, kaempheride, kaempferol, luteolin, 7-*O*-apigenin glucoside, 7-*O*-luteolin glucoside, piperitone were from Extrasynthese (Lyon, France). Camphor, caryophyllene oxide, α-cubebene, *α*-terpineol, terpinen-4-ol, thujone, verbenone were purchased from Fluka (Steinheim, Germany). All authentic compounds used have an average purity of 95%.

2.2. Plant material

Leaves of air dried samples (harvest 2010) of dittany (*Origanum dictamnus* L.), lemon balm (*Melissa officinalis* L.), rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia officinalis* L.) were used. In the case of chamomile (*Matricaria recutita* L.) flowers' heads were used. Certified plant material, except of dittany, were kindly provided by Aetoloakarnania's Rural Cooperative of Aromatic, Pharmaceutical and Energy Plant Cultivators -ASKAFEFA (Agrinio, West Greece). Dittany was obtained from a local market in Rethymno (Crete, South Greece). Identification of dittany was performed by Assistant Professor of Systematic Botany Panayiotis Trigas and a voucher specimen is deposited in the herbarium of Agricultural University of Athens (Athens, Greece), with a reference number 8625 (ACA). Dry plant material was stored at −20 °C until use.

2.3. Extraction procedure

Plants were subjected to sequential extraction with petroleum ether (PE), diethyl ether (DE) and methanol (ME). PE and DE extraction was performed in an ultrasound water bath Sonorex Super RK 255H type (Berlin, Germany), at a fixed frequency of 35 kHz, at 25 °C for 15 min. In brief, in a sample flask 100 mL of PE were added to 6 g of plant material and after sonication the supernatant was collected and filtered through polytetrafluoroethylene filter (PTFE) with a 0.45 μm pore size (Machery-Nagel, Düren, Germany). Six sonication steps were performed by adding 100 mL of PE to the plant residue and filtrates were merged (600 mL). Then, in the plant residue, the same 6-step procedure was repeated having DE as the solvent system. Finally, the plant residue was transferred in a 2000 mL beaker and 600 mL of ME was added. The mixture was stirred by a magnetic bar (1000 rpm) overnight, in the dark and it was then filtered through a PTFE filter. Organic extracts were concentrated under vacuum to a volume of 5 mL using a rotary evaporator type Heidolph Laborota 4000 Efficient (Schwabach, Germany). They were then further evaporated to dryness by a gentle flow of nitrogen and kept at −20 °C until analysis.

2.4. Gas chromatography analysis of PE extracts

Identification of compounds was performed on a Hewlett Packard 5890 II gas chromatographer (GC) (Palo Alto, CA, USA) equipped with an Rtx-5MS capillary column (30 m × 0.25 mm; film thickness, 0.25 μm) and coupled to a mass spectrometer (MS) detector Hewlett Packard 5972. Chromatographic conditions performed as described by Rodríquez-Solana et al. (2014). Injector and MS transfer line temperatures were set at 220 °C and 290 °C, respectively. Column temperature raised from 60 °C to 250 °C at a rate of 3 °C/min. Flow rate of helium was 1 mL/min. Samples of 1 μL were injected manually in the splitless mode. Mass spectra were obtained in electronic impact mode at 70 eV. Identification of compounds was based on comparison of their relative retention time and mass spectra with those of authentic standards. For peaks with no available reference standard, tentative identification of the compounds was based on the comparison of their relative retention time and mass spectra to those of NIST 98, Wiley 275, Adams 07 library data of the system and data available in the literature (Adams, 2007).

For quantification, extracts were analyzed using Hewlett-Packard 5890 II GC equipped with a flame ionization detector (FID) under the same conditions as above. For each compound of interest an individual response factor was calculated. In the cases where no standard samples were available, the response factor was used to quantify the analyte corresponding to commercial standards of similar molecular structure. Bicyclic hydrocarbon monotrpenes were quantified as α-pinene, monocyclic monoterpenes alcohols as terpine-4-ol, bicyclic monoterpenes alcohols as borneol, monocyclic monoterpene ketone as piperitone, bicyclic monoterpene ketones as camphor, spiroethers as 1,4-dioxaspiro[4.4]non-6-ene, bicyclic sesquitepenes as *β*-caryophyllene, tricyclic sesquiterpenes and sequiterpenoids as α-cubebene, and bisabolol and bisabolene derivatives as α-bisabolol. All measurements were performed in triplicates.

2.5. Liquid chromatography analysis of DE and ME extracts

Phenolic compounds found in the extracts were analyzed with a Shimatzu LC/MS-2010A, equipped with a LC-10ADvp binary pump, a DGU-14A degasser, a SIL-10ADvp autosampler (volume injected: 20 μL), a SPD-M10Avp diode array detector (DAD) and a quadrupole mass spectrometer with electrospray interface operating in negative mode. All hardware components were controlled by the Shimatzu software, version 3.40.307.

Dry plant extracts were dissolved in ME to a final concentration of 2 mg/mL for ME and 10 mg/mL for DE extracts, and filtered through a PTFE filter with a 0.20 μm pore size. Separation was achieved on a

Download English Version:

https://daneshyari.com/en/article/5761838

Download Persian Version:

https://daneshyari.com/article/5761838

Daneshyari.com