Journal of Applied Research on Medicinal and Aromatic Plants 6 (2017) 31–40

Contents lists available at ScienceDirect

Journal of Applied Research on Medicinal and Aromatic Plants

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

Optimised extraction of antioxidant polyphenols from *Satureja thymbra* using newly designed glycerol-based natural low-transition temperature mixtures (LTTMs)

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ARTICLE INFO

Article history:
Received 18 May 2016
Received in revised form
29 December 2016
Accepted 11 January 2017
Available online 23 January 2017

Keywords:
Antioxidants
Low-transition temperature mixtures
Extraction kinetics
Flavonoids
Polyphenols
Satureja thymbra

ABSTRACT

The medicinal plant *Satureja thymbra* (pink savory) has been chosen to study the capacity of three newly designed glycerol-based, natural low-transition temperature mixtures (LTTMs) to extract polyphenolic antioxidants. First, it was assessed the effect of water concentration (C_W) and the liquid-to-solid ratio ($R_{L/S}$), by deploying response surface methodology (Box-Behnken design). As a further step, kinetics was investigated to examine the effect of temperature. It was found that the optimum C_W varied from 54.8 to 63.8% (v/v) and $R_{L/S}$ from 29.5 to 36.2 mL g^{-1}. Under optimised conditions, all three LTTMs displayed apparent anti-Arrhenius kinetics over a temperature ranging from 40 to 80 °C, evidencing peculiar extraction behaviour. The investigation of the polyphenolic composition of the extracts obtained by liquid chromatography-diode array-mass spectrometry showed that the major constituents were apigenin, luteolin, quercetin and rosmarinic acid derivatives, but none of the LTTMs exhibited notable selectivity. However, significant differences were seen with regard to the antiradical activity and reducing power of the extracts, as extract obtained with LTTM1 displayed consistently strong effects, whereas the extract obtained with LTTM3 had rather questionable efficiency.

1. Introduction

Contemporary trends for the recovery of bioactive compounds from natural sources have shifted industrial demands towards sustainable processes, to eliminate the environmental risks posed by chemical procedures and develop cost-effective and eco-friendly extraction methodologies. This orientation would embrace strategies towards high efficiency and no generation of further waste streams and need for reuse/recycling of the solvents used, which is inevitably associated with higher operating costs and energy consumption. In this line, the search for alternative green extraction media is of undisputed importance. Organic solvents are regularly required to ensure a convenient separation of products, but conventional low boiling point solvents raise serious concerns related with storage, flammability and worker exposure. As a consequence, the chemical industry is in the search for innovative and safe media, with the aim of maximizing the sustainability and safety of extraction processes (Gu and Jérôme, 2013).

Low-transition temperature mixtures (LTTMs), also known as deep eutectic solvents (DESs) are a novel class of eco-friendly liquids composed of bioorganic molecules, such as a polyol serving as the hydrogen bond donor (HBD), and an organic salt, which is the hydrogen bond acceptor (HBA) (Abbott et al., 2011; Durand et al., 2016). LTTMs possess particularly attractive attributes, including lack of toxicity, low cost, negligible vapour pressure, tunability, etc., which make them ideal extraction media surpassing the qualities and limitations associated with similar materials, such as conventional organic solvents and ionic liquids (Wagle et al., 2014). LTTMs are generally less expensive, synthetically simple (bulk commodity chemicals can be used) and biodegradable. In this regard, LTTMs are compatible with foods, pharmaceuticals and cosmetics, but also environmentally benign. The ability to tailor the physicochemical properties of LTTMs is central to customising their extraction behaviour, as a number of essential parameters (viscosity, polarity, surface tension, hydrogen bonding) that play significant roles

Abbreviations: AAE, ascorbic acid equivalents; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; GAE, gallic acid equivalents; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; LTTMs, low-transition temperature mixtures; TPTZ, 2,4,6-tripyridyl-s-triazine.

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http://dx.doi.org/10.1016/j.jarmap.2017.01.002
2214-7861/

Nomenclature

A_{AR}	Antiradical activity (μmol DPPH g^{1})
C_w	Water concentration (%, v/v)
D_e	Effective diffusion coefficient ($m^2\ s^{-1}$)
E_a	Activation energy ($kJ\ mol^{-1}$)
h	Initial extraction rate ($mg\ g^{-1}\ min^{-1}$)
k	Extraction rate constant ($g\ mg^{-1}\ min^{-1}$)
k_0	Temperature-independent factor (min^{-1})
P_R	Reducing power (μmol AAE g^{-1})
$R_{L/S}$	Liquid-to-solid ratio ($mL\ g^{-1}$)
t	Time (min)
T	Temperature (°C or K)
Y_{TP}	Yield in total polyphenols (mg GAE g^{-1})
$Y_{TP(s)}$	Yield in total polyphenols at saturation (mg GAE g^{-1})

in mass transport properties governing an extraction process, may be effectively regulated (Dai et al., 2015).

Several natural products originating from plant sources have been regarded as potential drugs and nowadays the search for alternative therapies has led to the testing of numerous medicinal herbs that may improve or even eliminate symptoms of a spectrum of diseases. Among the various medicinal herbs, *Satureja* species (e.g *S. hortensis*, *S. montana*, *S. thymbra*, etc.), which belong to the Lamiaceae family, are used as a pleasant spice, food additive, as well as an herbal tea. Extracts from aerial parts of *Satureja* plants have been claimed to possess a multitude of biological properties, such as antioxidant, anti-inflammatory, analgesic and antiproliferative activities, but also antifungal and antibacterial and antiviral effects (Babajafari et al., 2015).

Recent studies pertaining to the phytochemical composition of *Satureja* aerial parts revealed the existence of an array of pharmacologically and nutritionally important components, including carvacrol, flavonoids like apigenin, naringenin, luteolin, eriodictyol, etc., and phenolic acids (Saeidnia et al., 2016). However, although there is a large amount of data on the polyphenolic composition of a number of *Satureja* species (Tepe and Cilkiz, 2015), similar information for *S. thymbra* is rather particularly limited. This being the case, the study presented herein was undertaken to i) synthesise novel LTTMs based on biomolecules, ii) optimise the water content for efficient extraction of antioxidant phytochemicals from aerial parts of *S. thymbra*, iii) test the effect of temperature on the extraction process and iv) obtain some information regarding the selectivity of the LTTMs tested, the polyphenolic composition and antioxidant properties of the extracts.

2. Materials and methods

2.1. Chemicals

Glycerol (>99%) was from Fisher Scientific (New Jersey, U.S.A.). All solvents used for chromatographic purposes were HPLC grade. Folin-Ciocalteu phenol reagent was from Fluka (Steinheim, Germany). Gallic acid, ascorbic acid, 2,4,6-tripyridyl-*s*-triazine (TPTZ) and 2,2-diphenyl-picrylhydrazyl (DPPH•) stable radical were from Sigma Chemical Co (St. Louis, MO, U.S.A.). Choline chloride was from (>98%) was from Alfa Aesar (Karlsruhe, Germany). Sodium acetate trihydrate and trisodium citrate dihydrate were from Penta (Praha, Czech Republic).

Table 1
Experimental values and coded levels of the independent variables used for the experimental design.

Independent variables	Code units	Coded variable level		
		−1	0	1
C_w (v/v, %)	X_1	10	45	80
$R_{L/S}$ ($mL\ g^{-1}$)	X_2	10	55	100

2.2. Plant material

Aerial parts of *S. thymbra* (Lamiaceae) were provided by the MAICh Herbarium (Chania, Greece), where a voucher specimen was deposited (9786 MAIC). The plant tissue was left to dry in a dark chamber for seven days (moisture level <17%) and then ground in a domestic blender to yield a powder with approximate mean particle diameter of 1 mm. The powder was stored in a stoppered glass vial at room temperature, in the dark, until used.

2.3. Preparation of the LTTMs

LTTMs were synthesised following standard procedures, as previously described (Dai et al., 2015). Briefly, glycerol (HBD) was mixed with each salt (choline chloride, sodium acetate trihydrate, trisodium citrate dihydrate), which served as HBA, at predetermined molar ratios. The mixtures were heated (usually at 60–80 °C) under stirring (600 rpm) until the formation of a perfectly transparent liquid. LTTMs were stored in sealed glass vials in the dark, at room temperature.

2.4. Experimental design and extraction procedure

To investigate the effect of the amount of water (C_w) and the liquid-to-solid ratio ($R_{L/S}$) on the yield of total polyphenol extraction (Y_{TP}), a response surface methodology was employed by implementing a central composite (Box-Behnken) design, based on previous studies (Katsampa et al., 2015). The levels of the two independent variables used (C_w and $R_{L/S}$) selected are analytically presented in Table 1. Extractions were carried out in 50 mL of solvent, composed of various combinations of each of the LTTMs with water and at various $R_{L/S}$, as dictated by the experimental design, and the response (Y_{TP}) at each design point was measured (Table 2). Analysis of variance (ANOVA) was used to assess the statistical significance of the mathematical models obtained. Insignificant dependent terms ($p > 0.05$) were omitted from the models (Table 3). The optimisation outcome was visualised through contour plots (Fig. 1), which were obtained using the fitted models. All extractions were performed under continuous stirring at 600 rpm and 50 °C, in a temperature-controlled oil bath (YellowLine MST Basic C, Richmond, VA, U.S.A.), for 200 min.

2.5. Kinetic assay and determination of diffusivity (D_e) and activation energy (E_a)

Extractions were carried out as described above, under optimised conditions (Table 4) and sampling was accomplished at predetermined intervals (5, 10, 20, 30, 50, 90, 120 and 200 min), to calculate Y_{TP}. Then Y_{TP} was plotted against time (t) and model fitting was performed with non-linear regression (Fig. 2, left plots). The second-order model was obtained after plotting $1/Y_{TP}$ as a function of t (Fig. 2, right plots), according to the following equation (Apostolakis et al., 2014):

$$\frac{t}{Y_{TP(t)}} = \frac{1}{kY_{TP(s)}^2} + \frac{t}{Y_{TP(s)}} \quad (1)$$