Journal of Applied Research on Medicinal and Aromatic Plants 3 (2016) 120–127

Contents lists available at ScienceDirect

Journal of Applied Research on Medicinal and Aromatic Plants

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

Novel lactic acid-based natural deep eutectic solvents: Efficiency in the ultrasound-assisted extraction of antioxidant polyphenols from common native Greek medicinal plants

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ARTICLE INFO

Article history:
Received 10 December 2015
Received in revised form 4 February 2016
Accepted 13 March 2016
Available online 25 March 2016

In memory of Dr. Panagiotis Kefalas.

Keywords:
Antioxidants
NADES
Medicinal plants
Polyphenols
Ultrasound-assisted extraction

ABSTRACT

Selected native Greek medicinal plants, including dittany, fennel, marjoram, mint and sage, were used to test the efficiency of some novel lactic acid-based natural deep eutectic solvents (NADES) to extract polyphenolic compounds. Extractions were performed under ultrasonication and the eutectic mixtures, tested as 80% (v/v) aqueous solutions, were lactic acid:choline chloride, lactic acid:sodium acetate, lactic acid:ammonium acetate and lactic acid:glycine:water, with corresponding molar ratios of 3:1, 3:1, 3:1 and 3:1:3. The three latter NADES are reported for the first time. Water and 60% (v/v) aqueous ethanol were also used as control solvents. The results obtained evidenced that lactic acid:glycine:water exhibited high efficiency, but in some instances lactic acid:sodium acetate and lactic acid:ammonium acetate were equally efficient. The data also suggested that extracts with high polyphenol concentration may also possess higher antiradical activity and reducing power. The NADES tested are non-toxic, renewable and exceptionally efficient solvents for polyphenol recovery from medicinal plants. The findings of this study were interpreted on the ground of assumptions regarding the polarity of the NADES tested.

1. Introduction

The Mediterranean flora displays high biodiversity and embraces a richness of native aromatic plants, many of which have been demonstrated to exhibit pharmacological potential. There is thus a global trend in the application of recognized potent herbal plants as pharmaceuticals, cosmetic bioactives and food ingredients, in the light of numerous findings, which have shown that the medical properties of a plethora of herbal formulations are often attributed to polyphenolic compounds (Farzaneh and Carvalho, 2015). Polyphenols have gained a great attention due to their multiple beneficial effects on human health (Li et al., 2014), such as antioxidant activity, antimicrobial activity, as well as chemoprotective potency and for this reason there have been to date a large number of studies pertaining to the exploitation of polyphenol-bearing medicinal plants. The interest has been focused on the development of efficient and cost-effective downstream processes, which aim at producing commodities on the basis of either crude or purified extracts.

A key factor involved in such a process is the effective recovery of polyphenols from the plant matrix, which is mostly carried out by solid-liquid extraction. Among other parameters, the selection of an appropriate solvent is of utmost importance and largely defines yield and composition of the extracts produced. Solvents play a major role in the chemical industry, as they are essential for many applications, and the most commonly used solvents to date have been of petrochemical origin (Bergez-Lacoste et al., 2014). Conventional solvents are often highly flammable and toxic, and their manufacture depends on fossil resources. Thus numerous investigations are currently focusing on the replacement of hazardous solvents with more environment-friendly alternatives. Biomass-derived chemicals offer promising opportunities in the search for eco-friendly, 'sustainable', or 'green' solvents and an array of materials have been considered in this regard.

The pioneering study of Abbott et al. (2003) presented a new type of solvents, which are formed by mixing solid materials with high melting points. Thereby, a new solvent system, termed "deep eutectic solvents", that consists of natural and renewable starting materials was introduced. In such mixtures, hydrogen bonding interactions are the main driving force of the eutectic phenomenon

Abbreviations: AAE, ascorbic acid equivalents; ChCl, choline chloride; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DT, dittany; FN, fennel; GAE, gallic acid equivalents; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; MJ, marjoram; MT, mint; NADES, natural deep eutectic solvents; RtE, rutin equivalents; SG, sage; TPTZ, 2,4,6-tripyridyl-*s*-triazine.

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http://dx.doi.org/10.1016/j.jarmap.2016.03.003
2214-7861/

Nomenclature

A_{AR}	Antiradical activity (μmol DPPH g^{-1})
AED	Acoustic energy density (W L^{-1})
C_{TFn}	Total flavonoid concentration (mg RtE L^{-1})
C_{TP}	Total polyphenol concentration (mg GAE L^{-1})
P_R	Reducing power (μmol AAE g^{-1})
$R_{L/S}$	Liquid-to-solid ratio (mL g^{-1})
T	Temperature (°C)
Y_{TFn}	Yield in total flavonoids (mg RtE g^{-1})
Y_{TP}	Yield in total polyphenols (mg GAE g^{-1})

and the study on hydrogen-bond donor (HBD)—hydrogen-bond acceptor (HBA) combinations enables tailoring the nature, physical properties, and phase behaviour of eutectic mixtures (EMs). The most commonly used HBA is the salt choline chloride (melting point 302 °C) and its combination with a suitable HBD (e.g., polyols) produces eutectic mixtures that are liquid at ambient temperature and have unusual solvent properties (Francisco et al., 2013).

Although there have been some recent reports on the extraction of various bioactive substances from plant material using NADES (Cui et al., 2015; Dai et al., 2013a; Qi et al., 2015), there are countless combinations of natural constituents that could be used and therefore the synthesis of NADES and the study of their extraction efficiency is imminent. In such a framework, this examination had as a scope the generation of novel NADES, based on low-cost, non-toxic natural substances, including L-lactic acid and salts of acetic acid, as well as the natural aminoacid glycine. The capacity of these novel NADES to recover polyphenolic compounds was evaluated using several common native Greek medicinal plants, with variable polyphenolic composition. The estimation of the antioxidant activity served as an additional means of assessing the properties of the extracts obtained.

2. Materials and methods

2.1. Chemicals

L-Lactic acid was from Panreac (Barcelona, Spain). Gallic acid, glycine, rutin (quercetin 3-*O*-rutinoside), Folin-Ciocalteu reagent, ascorbic acid, 2,4,6-tripyridyl-*s*-triazine (TPTZ) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were from Sigma-Aldrich (St. Louis, MO, U.S.A.). Sodium-potassium tartrate tetrahydrate, ammonium acetate and sodium acetate trihydrate were from Penta (Praha, Czech Republic). Choline chloride (>98%) was from Alfa Aesar (Karlsruhe, Germany). Ferric chloride hexahydrate and anhydrous aluminium chloride were from Acros Organics (New Jersey, U.S.A.). Absolute ethanol and acetic acid were from Fisher Scientific (New Jersey, U.S.A.).

2.2. Plant material

All samples were purchased from a local store of certified botanicals (Myrina, Lemnos). The samples were dittany (*Origanum dictamnus*), fennel (*Foeniculum vulgare*), marjoram (*Origanum majorana*), sage (*Salvia officinalis*) and mint (*Mentha spicata*), originating from various areas of Greece. All plant material was pulverised in a laboratory mill (Tristar, Tilburg, The Netherlands) to give powders with approximate mean particle diameter <0.5 mm. The pulverised material was kept in sealed plastic tubes, in the dark, at ambient temperature for no longer than 7 days.

2.3. Preparation of the NADES

The synthesis of all NADES tested was based on previously reported methodologies (Dai et al., 2013b, 2015). Briefly, L-lactic acid (hydrogen bond donor – HBD) was mixed with choline chloride, sodium acetate, ammonium acetate and glycine (hydrogen bond acceptors – HBA) at predetermined molar ratios and the mixtures were mildly heated under stirring, until a perfectly transparent liquid was formed. NADES were kept in sealed glass vials in the dark, at ambient temperature. The codes of the NADES used in this study, along with details regarding their synthesis are analytically given in Table 1.

2.4. Batch ultrasound-assisted extraction process

All NADES were used as 80% (v/v) aqueous solutions. Incorporation of 20% water was deemed necessary, to reduce the viscosity of the NADES and facilitate polyphenol diffusion (Karakashov et al., 2015). Extractions were carried out according to a previously described methodology (Katsampa et al., 2015). Briefly, an amount of 0.1 g of each pulverised material was placed in a 15-mL screw-cup tube together with 10 mL of NADES solution, to give a liquid-to-solid ratio ($R_{L/S}$) of 100 mL g^{-1} (Blidi et al., 2015). Deionised water and 60% (v/v) aqueous ethanol were also used for comparison. The mixtures were shaken vigorously manually for a few seconds to form slurry and then extracted at 80 °C for 90 min, in a temperature-controlled, sonication bath (Elma P70, Singer, Germany), at a sonication power of 140 W, a frequency of 37 kHz, and an acoustic energy density (AED) of 35 W L^{-1} (Katsampa et al., 2015). Following extraction, samples were centrifuged at 15,000 rpm for 10 min in a Table centrifuge (Hermle, Wehingen, Germany), and diluted 1:20 with distilled water. This solution was used for all analyses.

2.5. Determination of total polyphenol yield (Y_{TP})

A previously reported protocol was used (Karakashov et al., 2015). An aliquot of 0.78 mL of distilled water, 0.02 mL of sample and 0.05 mL of Folin-Ciocalteu reagent were mixed. After exactly 1 min, 0.15 mL of aqueous sodium carbonate 20% was added, and the mixture was left at room temperature in the dark, for 60 min. The absorbance was read at 750 nm (A_{750}) and the total polyphenol concentration (C_{TP}) was calculated from a calibration curve, using gallic acid as a standard (25–500 mg L^{-1}). Yield in total polyphenols (Y_{TP}) was determined as mg gallic acid equivalents (GAE) per g of dry weight (dw).

2.6. Determination of total flavonoid yield (Y_{TFn})

A previously published methodology was employed (Makris, 2015). An aliquot of 0.25 mL sample was mixed with 0.75 mL $AlCl_3$ reagent [0.16% (w/v) $AlCl_3$ and 5% (v/v) acetic acid in methanol] and allowed to stand for 30 min, at room temperature. The absorbance was obtained at 415 nm (A_{415}) and the total flavonoid concentration (C_{TFn}) was calculated from a calibration curve, constructed with rutin (quercetin 3-*O*-rutinoside) as the calibration standard (10–160 mg L^{-1}). Yield in total flavonoids (Y_{TFn}) was expressed as mg rutin equivalents (RtE) per g of dry weight.

2.7. Determination of the reducing power (P_R)

A methodology reported previously was used (Karakashov et al., 2015). Sample (0.05 mL) was mixed thoroughly with 0.05 mL $FeCl_3$ solution (4 mM in 0.05 M HCl), and incubated for 30 min in a water bath at 37 °C. Following this, 0.9 mL TPTZ solution (1 mM in 0.05 M HCl) was added, and the absorbance was recorded at 620 nm after