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Ionic liquid-supported solid–liquid extraction of bioactive alkaloids. III. Ionic liquid regeneration and glaucine recovery from ionic liquid-aqueous crude extract of *Glaucium flavum* Cr. (Papaveraceae)

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ABSTRACT

In continuation of a research project aiming at introducing ionic liquids (ILs) as an alternative to the widely applied for the recovery of natural products of industrial interest conventional molecular solvents, we developed a concise procedure for isolation of the biologically active alkaloid *S*-(+)-glaucine from IL-based aqueous crude extract. To this end, a comparative study of the behavior of 1 M [C_4C_1im][Ace]-aqueous solution and methanol in a series of consecutive extractions with the same extractant was conducted. The results obtained proved the better performance of the IL-based system in the solid–liquid extraction step, since the latter showed constantly higher extraction efficiency (ca. 35% enhanced) compared to methanol. The above procedure allows glaucine accumulation from at least ten successive extractions, while simultaneously reduces the total solid–liquid ratio from 1:40 to 1:7.2, without loss of efficiency. Furthermore, the loss of IL into the matrix pores after extraction was also considered, suggesting the need for IL recycling by posttreatment of the residual biomass. To recover glaucine from the crude IL-based aqueous extract, a series of non-miscible with water molecular solvents were tested. As a result, optimal conditions for quantitative extraction into chloroform were found, from which, after solvent removal and subsequent crystallization from ethanol, the target compound was isolated as a hydrobromide salt, the latter being the marketed form of glaucine.

1. Introduction

Plants, animals, and microorganisms represent a sustainable source of natural products known to be useful to human beings for millennia. Indeed, diverse natural species are still the main source of ideas toward the development of new drugs, functional foods, and food additives [1]. Among the others, plant alkaloids can be considered to be of an immense importance, since despite they cover about 15% of the known natural substances, they represent nearly 50% of the plant products of pharmaceutical interest. The manufacturing of secondary metabolites from their native sources proceeds according to well-established procedures [2,3], which usually begin with exhaustive extraction with molecular solvents, e.g., saturated hydrocarbons, alcohols, halogenoalkanes, etc., and followed by additional chemical treatment of the crude extracts in order for the compounds of interest to be isolated in a pure form. These procedures are multistage, laborious, time and energy consuming, and require complicated equipment. Moreover, the organic solvents employed in the production of natural products do not always provide exhaustive extraction and are flammable, volatile, and toxic, which is in a contradiction with the universally accepted nowadays 12 principles of the green chemistry [4]. Thus, the need of extractants of improved characteristics from safety, ecological, toxicological and technological standpoint can be suggested [5,6].

Ionic liquids (ILs) are promising candidates that could meet the above mentioned requirements. ILs, also named as designers solvents, received a significant attention from the scientific community in the last two decades [7]. Consisting entirely of ions (usually non-symmetrical charge-stabilized organic cation and inorganic or organic anion) they are liquids at ambient temperature and display a wide range of unique properties, such as negligible vapor pressure, non-flammability at ambient conditions, high thermal stability, and low chemical reactivity. These unique properties suggest ILs as potential substituents of commonly employed in different processes volatile, flammable and toxic organic solvents [8]. Moreover, the versatility of possible ion combinations, each of them hypothetically resulting in a new IL, allows their physicochemical properties, e.g. density, viscosity, polarity, miscibility

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with other common solvents, etc. to be fine "tuned" simply by careful selection of the ions [9–12], thereby allowing "IL tailoring" for a particular application.

To date, ILs have been successfully introduced instead of molecular solvents in numerous fields such as synthesis [13], catalysis [14], electrochemistry [15], and analytical chemistry [16], to name just a few. Additionally, ILs have proved to be efficient extractants in the recovery of a wide variety of value-added chemicals [5,6], in all cases providing enhanced extraction yields and significant reduction of the extraction times and solvent consumption. The latter was believed to be due to the stronger dissolving power of ILs, but it was recently shown by us [17] that their role in the solid–liquid extraction processes is not limited only to the enhanced interactions provided by the ions, but can be rather attributed to the pronounced solvent–matrix interactions leading to a plant matrix disruption and permeability modification [17]. A range of techniques possessing specific requirements and certain advantages compared to the rest can be employed in the IL-assisted extractions of natural products, which include simple batch extraction (HRE) [18–20], microwave-assisted extraction (IL-MAE) [21–23], ultrasound-assisted extraction (IL-UAE) [24–26], IL-assisted ultrahigh pressure extraction (IL-UPE) [27], and IL-based negative pressure cavitation-assisted extraction (IL-NPCE) [28]. However, due to their limited applicability in a scale-up processes, these techniques have been mainly applied in a laboratory scale to ensure exhaustive extraction, in order for novel analytical methods to be developed, and, in majority of the cases, neither attempts for ILs recycling – an important issue that addresses the economics of their use have been done, nor the possible ways for solutes of interest to be isolated in pure form after the solid–liquid extraction step have been examined. Indeed, a short retrospection of the recent literature shows that these issues are studied to a very limited extent, and that the methods employed depend on the specific properties of both ILs and solutes to be extracted. Particularly, an anti-solvent induced precipitation proved successful for isolation of neutral compounds [29] and hydrodistillation for the recovery of volatile compounds [30]. Additionally, back-extraction with organic solvents [18–20], partitioning in IL-based aqueous two-phase systems (IL-ATPS) [31–33], and in some cases, ion exchange resin [34] or resin for selective trapping [35] had given satisfactory results. Although some achievements have been done in this direction, additional work is necessary in order to provide useful information for the initial sizing and economic evaluation of the IL-assisted extraction process in a commercial scale.

In continuation of a research project aiming at introducing ILs as an alternative to the conventional molecular solvents, widely applied for the recovery of natural products of industrial interest [17,18], herein we report the development of a concise procedure for isolation of the biologically active alkaloid *S*-(+)-glaucine {[36–40], Fig. 1} from IL-aqueous crude extract. To achieve this, we initially performed a comparative study on the extraction ability of 1 M [C_4C_1im][Ace]-aqueous solution and methanol, being the solvent for the production of glaucine, in a batch regime. The results obtained proved the better performance of the IL-based system, since the latter showed constantly higher extraction efficiency (ca. 35% enhanced) compared to methanol. Moreover, the above mentioned procedure allows glaucine accumulation from at least ten successive extractions, while simultaneously reduces the total solid–liquid ratio without loss of efficiency. To recover glaucine from the crude IL-based aqueous extract, a series of non-miscible with water molecular solvents were tested and optimal conditions for quantitative extraction into chloroform were found. Finally, the target compound was isolated as a hydrobromide salt, the latter being the marketed form of glaucine.

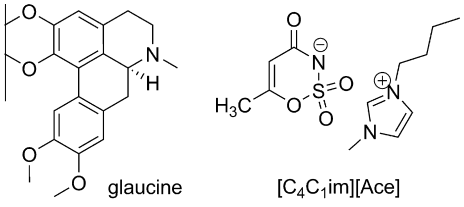

Fig. 1. Structures of glaucine and [C_4C_1im][Ace].

2. Materials and methods

2.1. Chemicals

All chemicals used in this study were purchased from Sigma–Aldrich (FOT, Bulgaria). The organic solvents were of analytical grade and acetonitrile used for HPLC analysis was of chromatographic grade. The IL used for extraction experiments was 1-butyl-3-methylimidazolium acesulfamate {[C_4C_1im][Ace], Fig. 1} and was synthesized, purified and characterized by the authors according to a recently published procedures for the synthesis of hydrophilic ILs [11]. Its structure and purity was unequivocally proven by means of ^{1}H and ^{13}C NMR spectral analysis [18]. Silver nitrate test showed no residual chloride anions.

Aerial parts of plant material of *Glaucium flavum* Cr. and standard sample of glaucine were obtained from the relevant Laboratory of Natural Products at the Bulgarian Academy of Science. The plant material was further grinded, and in order to reduce the moisture content, it was dried under vacuum prior to extraction. The particle size extracted was 0.25–0.40 mm. The same batch of sample was used through this study.

2.2. Apparatus, analysis and calculations

Glaucine quantification was carried out by means of reverse phase high performance liquid chromatographic analyses (RP-HPLC), performed on a GBC liquid chromatography system, equipped with a LC 1100 HPLC pump, a variable LC 1200 UV/Vis detector, a LC 1431 system organizer, an injector with a 20 μL loop and N2000 software for data treatment. A ZORBAX Extend-C18 (150 × 4.6 mm i.d., 5 μm) was used as an analytical column. The mobile phase was a mixture of 0.1% triethylamine aqueous solution and acetonitrile (50:50) delivered at a flow rate of 1 mL/min [17]. The UV detection wavelength was set up at 280 nm, where aporphine alkaloids have an optimum absorption. Each injection volume was 20 μL and the column temperature was ambient. Under these conditions the target alkaloid glaucine was baseline separated and its peak was symmetrical. The peak identification was achieved by a comparison of its retention time with the corresponding peak in a standard solution, and glaucine concentration was calculated according to a previously developed relationship. For all analysis, aliquots were taken and were diluted with a certain volume of acetonitrile/water mixture, in order to fit into the linear range of the standard curve, then were filtered through a 0.45 μm microporous membrane prior to analysis and were directly injected into the HPLC apparatus. All HPLC analyses were performed in a triplicate and the mean value was adopted.

The structures of both [C_4C_1im][Ace] and glaucine were proved by means of NMR spectral analyses, obtained with a Bruker DRX 250 NMR spectrometer operating at 250.13/62.5 MHz for ^{1}H and ^{13}C, respectively. The NMR spectra were consistent with that previously reported in the literature [18]. The IR analyses were carried out on Agilent Cary 630 FTIR spectrophotometer equipped with diamond ATR-1 bounce.

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