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Ionic liquid based ultrasonic-assisted extraction of forskolin from *Coleus forskohlii* roots

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ABSTRACT

Ionic liquid based ultrasonic-assisted extraction (ILUAE) was successfully applied to extract forskolin (FSK) from *Coleus forskohlii* roots. Six ionic liquids with different cations and anions were initially investigated in this work from which tetramethyl guanidium lactate (TMGL) was selected for further studies using ultrasound-assisted extraction. Parameters of ultrasound extraction such as ultrasonic power, duty cycle and extraction time were optimized to get maximum extraction of FSK. The maximum extraction yield of 0.508 ± 0.006% (w/w) corresponding to an extraction efficiency of 87.4 ± 1.04% of FSK was obtained under the optimized conditions. ILUAE offered shorter extraction time (4 h) as compared to Soxhlet extraction (12 h) which indicated it to be a rapid, efficient, simple and green approach for extracting FSK from *Coleus forskohlii* roots.

1. Introduction

Coleus forskohlii is a perennial member of *Labiatae* family. It has been used as a herbal remedy to treat hypertension, congestive heart failure, intestinal disturbance, respiratory disorders, insomnia, convulsions, liver fatigue, glaucoma, Alzheimer's disease and for prevention of cancer metastases (Kavitha et al., 2010; Alasbahi and Melzig, 2010a; Alasbahi, and Melzig, 2010b; Suryanayanan and Pai, 1998). This has made *C. forskohlii* an attractive target for chemical and pharmacological studies. In recent times, it has been shown to possess significant anti-hypertensive, cardiotonic, antispasmodic, antioxidant and anti-HIV effects (Alasbahi and Melzig, 2010b; Suryanayanan and Pai, 1998; Maioli et al., 2010; Sabde et al., 2011; Yang et al., 2011). Many of the beneficial effects of *C. forskohlii* have been attributed to the pharmacological actions of forskolin (FSK), a major diterpene isolated from the root of *C. forskohlii* (Bhat et al., 1977). FSK is known to increase cyclic adenosine monophosphate (cAMP) and cAMP mediated functions by activating the enzyme adenylate cyclase.

Chemical synthesis of FSK is difficult and uneconomical. Corey and Jardine (1989) reported extraction from natural sources to be a better approach than chemical synthesis of FSK. Different techniques have been employed for the extraction of FSK from *C. forskohlii* roots such as heat reflux (Srivastava et al., 2002), hydrotropic extraction (Mishra and Gaikar, 2009), microwave assisted extraction (Devendra and Gaikar, 2010), enzyme assisted three phase partitioning (Harde and Singhal, 2012) and supercritical fluid extraction (Harde et al., 2013). Heat reflux extraction and maceration extraction techniques have drawbacks of requiring longer extraction time and relatively large volumes of solvents (Luque de Castro and Garcia-Ayuso, 1998). Supercritical fluid extraction method requires expensive high pressure equipment and may also require organic solvents as co-solvents for complete extraction of the bioactives (Mishra and Gaikar, 2009). Therefore, in recent years there is an increasing demand for new eco-friendly extraction techniques with shorter extraction time, maximum extraction efficiency and with reduced requirement of organic solvents.

In this study we report an ionic liquid based ultrasonic-assisted extraction (ILUAE) as an alternative method for the recovery of FSK. An ionic liquid (IL) is an organic salt in the liquid state that consists of an organic cation paired with an organic or inorganic anion. ILs are widely used as solvents because of their excellent properties such as good conductors of electricity, low vapor pressure, low combustibility, excellent thermal stability, wide liquid regions and favorable solvating properties for a range of polar and non-polar compounds (Yang and Dionysiou, 2004). IL based materials interact with analytes through anion exchange, hydrogen bonding and hydrophobic interaction (Wanigasekara et al., 2010).

The extraction of bioactive compounds from plants using ILs is promising as a green method, in reducing the environmental pollution and improving the selectivity and extraction yields of

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interesting compounds in sample pretreatment processes compared to conventional organic solvents. IL-based methodologies have been widely applied in food hygiene, drug testing, environment monitoring, biological analysis and other areas because of their capability of separating organic and inorganic compounds (Zhang et al., 2010). IL-assisted sample pretreatment techniques such as liquid-liquid extraction, liquid-phase microextraction, solid-phase microextraction and aqueous two-phase systems extraction have also been reported (Plechkova and Seddon, 2008). Many researchers have used ILs as solvents for the extraction of phytonutrients such polyphenolic compounds (Ma et al., 2010) and terpene lactones (Lu et al., 2008). Alkaloids including the aporphine alkaloids (N-nornuciferine, O-nornuciferine and nuciferine) from *Nelumbo nucifera* (Ma et al., 2010), the piperidine alkaloid piperine from *Piper nigrum* (van Rantwijk and Sheldon, 2007), the phenolic alkaloids liensinine, isoliensinine and neferine from *N. nucifera* (Wu et al., 2009) have all been reported to be extracted by using ILs.

Ultrasound-assisted extraction has been successfully used for the extraction of the bioactive constituents from the plant materials (Huang et al., 2011) due to advantages of high reproducibility, reduction in extraction time and minimum solvent consumption, temperature and energy output (Du et al., 2009). To the best of our knowledge, extraction of FSK from *C. forskohlii* roots with ILs as the solvent has not yet been reported in the literature. The objective of the present work was to develop an effective, rapid, green and environmental friendly IL based ultrasonic-assisted extraction (ILUAE) of FSK from *C. forskohlii* roots, and to compare it with conventional solvent extraction.

2. Materials and methods

2.1. Materials

C. forskohlii roots were procured from Salem, Tamil Nadu, India. Dried *C. forskohlii* roots were ground in a mill fitted with 18 mesh sieve to get powders with particle size below 1 mm and stored in an air tight container for further studies. Standard FSK was a gift sample from Medicinal and Natural Product Research Laboratory, Institute of Chemical Technology, Mumbai, India. Acetonitrile, lactic acid extrapure, *n*-butyl bromide, ethanolamine, acetic acid, formic acid and methanol were procured from S. D. Fine Chemicals limited, Mumbai, India. Pyridine and trifluoro acetic acid was procured from SiscoChem, Mumbai, India. 1-Methylimidazole, *n*-butyl chloride, sodium tetrafluoroborate, 1,1,3,3-tetramethyl guanidine were procured from Spectrochem, Mumbai, India. All the ionic liquids ([Bmim]Cl, [Bmim]Br, [Bmim]BF_4, [N-butyl pyridinium]BF_4, [2-hydoxyethyl ammonium] formate, [TMGL] lactate, where Bmim = 1-butyl-3-methylimidazolium, TMGL = Tetramethyl guanidium lactate) were synthesized in our laboratory (Dupont et al., 2004; Tshibangu et al., 2011; Law et al., 2006; Bicak, 2005; Gao et al., 2004). All the chemicals used in this study were of synthetic grade.

2.2. Optimization of parameters for IL based extraction of forskolin from C. forskohlii root

C. forskohlii root powder (5 g) was dispersed in 50 ml of ILs (0.25 M) prepared in deionized water by gentle stirring using a magnetic stirrer. The extraction was carried out for 2 h by gentle stirring with magnetic stirrer at 30 °C. The extract obtained after IL extraction was filtered to remove solid particles and quantified for the content of FSK using HPLC. The FSK yield was expressed as % w/w of *C. forskohlii* root powder. Extraction efficiency (%) of FSK was calculated on the basis of FSK content obtained by conventional solvent extraction.

Six different ILs ([Bmim]Cl, [Bmim]Br, [Bmim]BF_4, [N-butyl pyridinium]BF_4, [2-hydoxyethyl ammonium] formate, [TMGL] lactate) were used to screen the most suitable cation to extract FSK from *C. forskohlii* root at 0.25 M. The selected IL, tetramethyl guanidine (TMG) with different anions *viz.* TMG trifluoroacetate, TMG acetate, TMG formate and TMG lactate were used at 0.25 M to investigate the effect of the anion on the extraction efficiency of FSK. For maximum extraction of FSK, concentration of TMGL (0.25, 0.5, 0.75 and 1.0 M), temperature of extraction (30 °C to 50 °C), time of extraction (2–10 h) and substrate concentration (5–20%, w/v) were optimized. The process of extraction was done as described above in this section. The IL extracts were then estimated for the content of FSK. All the analyses were done in triplicates. Statistical analysis was done using Student's *t* test.

2.3. Optimization of parameters for IL based ultrasonic-assisted extraction (ILUAE) of FSK from C. forkohlii roots

In ILUAE, ultrasound pretreatment was given to *C. forskohlii* root slurry prior to IL extraction. A Branson sonifier 450 unit (Danbury, Connecticut, USA) with a maximum power of 400 W was used. The unit was a rectangular container (23.5 cm × 13.3 cm × 10.2 cm) to which 40 kHz transducers were annealed at the bottom. Dried *C. forkohlii* root powder (5 g) was mixed with TMGL (0.75 M) solution in a flask. The flask was then partially immersed in the ultrasonic bath which contained ice cold water to maintain temperature. Different ultrasonic parameters were studied to improve the extraction efficiency of FSK from *C. forkohlii roots*.

Optimization of power requirement for the maximum extraction of FSK was done by varying the power from 18 to 58 W for 10 min. Effect of duty cycle (30–70%) as a pretreatment on extraction of FSK was evaluated by dispersing 10% w/v of roots in 0.75 M TMGL at optimized power of 50 W for 10 min. Optimization of the extraction time (5–25 min) of sonication was carried out for maximum extraction of FSK. Ultrasonic pretreatment followed by IL extraction was performed under previously optimized conditions of TMGL concentration, extraction temperature, substrate concentration and time of extraction (0.75 M, 40 °C, 10% w/v and 4 h respectively). The extract was then quantified for FSK content.

2.4. Conventional solvent extraction

C. forskohlii root powder was extracted in a Soxhlet apparatus using methanol for 12 h. The extract was cooled and then concentrated by evaporating in rotary vacuum evaporator under reduced pressure at 50 °C (Buchi Rotavapor, R-124, Switzerland). The solvent was recovered from rotavac. The FSK yield was expressed as % w/w of *C. forskohlii* root powder.

2.5. Analytical determination

A Jasco HPLC system fitted with Zorbax eclipse XDB C_{18} column (5 μm × 4.6 mm × 250 mm) was used. The column was equilibrated with an acetonitrile–water (50:50) mixture as mobile phase at a flow rate of 1.5 ml/min. FSK was detected by measuring UV absorption at 217 nm (Mukherjee et al., 2000). Retention time of standard FSK was 9.7 min. Quantification of FSK was done by extrapolating the area under the curve to the concentration of standard FSK in the range of 0–0.7 mg/ml. The regression equation correlating the area under the curve (Y) with concentration of FSK (X) was $Y = 1909077.70X$.