Tropical Journal of Pharmaceutical Research August 2014; 13 (8): 1339-1345
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v13i8.20

Original Research Article

Ionic Liquid-Based Ultrasonic/Microwave-Assisted Extraction of Steroidal Saponins from *Dioscorea zingiberensis* C. H. Wright

Peng Wang[1], Chaoyang Ma[1], Shangwei Chen[2], Song Zhu[2], Zaixiang Lou[1] and Hongxin Wang[1]*

[1]State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, [2]State Key Laboratory of Food Science & Technology, No.1800 Lihu Avenue, Wuxi 214122, Jiangsu, PR China

For correspondence: **Email: wanghongxin2009@hotmail.com; **Tel:** (086)510-85917795; **Fax:** (086)510-85329276*

Received: 24 March 2014 Revised accepted: 5 July 2014

Abstract

Purpose: *To develop a more green, efficient and low cost process for the extraction of steroidal saponins from Dioscorea Zingiberensis C. H. Wright.*
Methods: *Six kinds of ionic liquids with different cations and anions were evaluated. In addition, the extraction parameters of the ionic liquid based ultrasonic/microwave extraction were studied. In order to quantify the total saponin content accurately, all steroidal saponins were converted into diosgenin and quantified with an appropriate HPLC procedure. IL-UMAE under optimal conditions was compared with heat reflux extraction (HRE) and ultrasonic assisted extraction (UAE).*
Results: *1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIm]BF4) solution at concentration of 0.5 mol L-1 was selected as extraction solvent. The optimum conditions were as follows: solvent to solid ratio 15:1, microwave power 500 W and extraction time 8 min. Under these optimal conditions, the highest extraction yield of diosgenin 10.24 ± 0.31 mg g-1 was obtained. It took only 8 min to obtain the 91.67 % content of diosgenin obtained by traditional HRE. Extracting on time was shortened from 120 min to 8 min.*
Conclusion: *Compared with traditional methods, IL-UMAE method uses Ionic liquid-solvent which greatly shortens the extraction time. IL-UMAE as a simple, effective and environmentally friendly approach shows a broad prospect for active ingredient extraction.*

Keywords: *Dioscorea zingiberensis Steroidal saponins, Ionic liquid, Ultrasonic, Microwave*

INTRODUCTION

The medicinal plant *Dioscorea zingiberensis* C. H. Wright is a famous traditional Chinese medicine (TCM). It is widely distributed in Shanxi, Hubei and Yunnan provinces of China [1]. It is the important plant resource for diosgenin, a starting material for the semi-synthesis of drugs of steroidal hormones [2]. Diosgenin is stored in plants in the form of saponins that are regarded as the major active components of *D. zingibiensis* [3]. In the last few years, saponins have attracted worldwide attention because of their rich variety of structures and functional activities [4]. For example, anti-thrombotic activity [5], protective effects against hyperlipidemia and oxidative stress [6], cytotoxic activity against the cancer [7], inhibiting platelet aggregation [8] were reported.

As steroidal saponins attracting more attention, more extraction techniques are developing to obtain better extraction efficiency. A variety of extraction techniques, such as Soxhlet extraction, maceration extraction (MA) [9] and heat reflux extraction (HRE) have been applied for extraction of effective components. But these extraction techniques have some drawbacks such as taking long time, high energy consumption, and large amount of organic solvent consumption. Therefore, ultrasonic/ microwave-assisted synergic extraction (UMAE) technique has been used in the field of saponins extraction. It shows short-time, high efficiency and other advantages [10,11] for extraction of active ingredients of natural products. What is more, we focused on the green solvent. In recent years, ionic liquid (IL) as a kind of environmentally friendly solvent has shown great potential for replacing conventional organic solvents in many field [12,13,14]. ILs are composed of varied cations and anions that are in the liquid state at room temperature, they have low vapor pressures, are non-flammability, miscible with various organic solvents [15], and offer multiple alternatives to conventional organic solvent system. They are recyclable, environmentally safe and can alleviate environmental pollution.

In this study, ILs were employed as green solvent to extract steroid saponins from *Dioscorea zingiberensis*. It is the combined application of ultrasonic/ microwave and ILs for efficient extraction of steroidal saponins from *Dioscorea zingiberensis* using a “green” method.

EXPERIMENTAL

Plant materials

Dioscorea zingiberensis C. H. Wright fresh rhizomes were supplied by Dongguan Jingyuan industrial investment Co., Ltd (Guangdong, China). They were collected in Shannxi Province, People's Republic of China, in March 2013 and were identified by Assistant Professor Deping Xu (School of Food Science and Technology, Jiangnan University, People's Republic of China). A voucher specimen (no. *Dioscorea zingiberensis* 201303) was assigned and sample has been deposited at the Department of Food Functional Component, School of Food Science and Technology, Jiangnan University, People's Republic of China. Fresh *Dioscorea zingiberensis* C. H. Wrightwere cut into pieces and placed in a drying oven at 80 °C for 5 min to kill enzymes. Thereafter, they were dried at 50 °C and crushed into powder.

Chemicals and reagents

Diosgenin standard was purchased from the Shanghai Yuanye Biological Technology Co, Ltd (Shanghai, China). The purity of standard compound was greater than 99 %. Acetonitrile of chromatographic grade was purchased from Sinopharm Chemical Reagent Co, Ltd (Shanghai, China). Ultrapure water used for solutions and dilutions was made by Millipore-Q® 3 (Massachusetts, USA). All ionic liquids were purchased from Lanzhou Institute of Chemical Physics (Gansu, China). Their basic information and representative physico-chemical properties are shown in Table 1. Petroleum ether and other reagents were analytical grade and were purchased from Sinopharm Chemical Reagent Co, Ltd (Shanghai, China).

Apparatus

A CW-2000 Ultrasonic-microwave synergistic extraction device was purchased from Shanghai Xintuo Analytical Instruments Co., Ltd (Shanghai, China) and used for extraction experiment. Ultrasonic power was rated at 50 W in the extraction process. Microwave could be varied between 300 W and 800 W. Extraction process was strictly monitored to prevent solution bumping.

The DL-360B ultrasonic apparatus was purchased from Shanghai Zhisun Instrument Co., Ltd (Shanghai, China) and used for ultrasound assisted extraction.

Table 1: Basic information of six ILs

ILs	Molecular Formula	Molecular Weight	State (25 °C)	Solubility in water
$[C_4MIm]Cl$	$C_8H_{15}N_2Cl$	174.67	Solid	Soluble
$[C_4MIm]Br$	$C_8H_{15}N_2Br$	219.12	Solid	Soluble
$[C_2MIm]Br$	$C_6H_{11}N_2Br$	197.07	Solid	Soluble
$[C_2MIm]BF_4$	$C_6H_{11}N_2BF_4$	197.97	Liquid	Miscible
$[C_3MIm]BF_4$	$C_7H_{13}N_2BF_4$	212.00	Liquid	Miscible
$[C_4MIm]BF_4$	$C_8H_{15}N_2BF_4$	226.02	Liquid	Miscible

The 1100 series HPLC system was purchased from Agilent (California, USA). It was equipped with a workstation, an online degasser, a column oven, an infusion pump, an auto sampler, and an ultraviolet detector. Chromatographic separation was performed on Grace Smart RP C18 column (5 µm, 4.6 × 250 mm. W. R. Grace & Co.-Conn, Columbia, Maryland, USA). All of the solvents prepared for HPLC were filtered through 0.22 µm microporous membrane. AR224CN electronic balance was purchased from Ohaus Instruments (Shanghai) Co., Ltd (Shanghai, China).

Ionic liquid-based ultrasonic/microwave-assisted extraction

According to the characteristics of the target component, many kinds of ionic liquids were selected and screened based on the diosgenin yield. Furthermore, different length alkyl chains of ILs were compared. Certain types of ionic liquids were prepared by accurately weighing a certain mass in a beaker. Then they were fully dissolved in deionized water, transferred to a volumetric flask and diluted to different concentrations. These ionic liquids were used for the extraction process. And diosgenin yield as an evaluation criterion clearly reflected their different extract abilities.

The whole extraction process was completed in an ultrasound-microwave synergistic extraction device. Steroid saponins were extracted by adding sample to a certain volume of the ionic liquid solution in a flask. Then extraction process was carried out by preset program with different parameters.

For maximize extraction yield, type of ionic liquid, liquid concentration, solvent to solid ratio, microwave power and extraction time were optimized.

Conventional extraction method

In order to investigate the performance of the IL-UMAE, conventional heat reflux extraction (HRE) and ultrasonic assisted extraction (UAE) were selected as controls for comparison. all the samples was carried out under the same conditions after the extraction process.

HRE: 1.0 g of plant powder (0.3 mm) was mixed with 25 mL of 70 % ethanol. The extraction was carried out at 60 °C for 1 h and the extraction was repeated two times.

UAE: 1.0 g sample was mixed with 25 mL of 70 % ethanol. The extraction was carried out at 50 °C for 40 min. Ultrasonic power was rated at 300 W.

Analysis and conditions

Diosgenin, the hydrolysate of steroidal saponins, is applied to quantify total steroid saponins extracted from *Dioscorea zingiberensis* C. H. Wright rhizomes. [16]

After extraction, extraction device started the cooling process and the extracts were filtered. The filtrate was mixed with hydrochloric acid to 2 mol L^{-1} acid solution and hydrolyzed for 3 h in 98 °C water bath at normal pressure. After cooling, hydrochloric acid solution was neutralized by Na_2CO_3 followed by filtration. The residue was washed with distilled water several times until filter liquor was colorless. The filtrate was dried and the residue was dried at 50 °C, and then extracted in a Soxhlet extractor for 3 h with petroleum ether. The solvent extract was concentrated to dryness by the rotary evaporator. Thereafter, the diosgenin was dissolved with methanol and filtered through 0.22 µm filters. The diosgenin was determinate by HPLC.

HPLC quantitative analysis for diosgenin was performed on the Agilent 1100 series. 10 µl of the sample was injected into C18 column at 30 °C, and the flow rate was kept at 1.0 mL min^{-1}. The detection wavelength was set at 209 nm. The mobile phase composition was acetonitrile-water (95:5, v/v). The regression equations and correlation coefficients (R^2) of diosgenin were determined and derived as y = 4418.6x – 41.792 (R^2 = 0.9999, 0.0993 mg mL^{-1}-1.5888 mg mL^{-1}). The content of diosgenin was used to evaluate each extraction method. The extraction yield of diosgenin was calculated as follows: Yield of diosgenin (mg g^{-1}) = diosgenin content / *Dioscorea zingiberensis* C. H. Wright weight.

RESULTS

Screening of ionic liquids

As shown in Fig 1(a), the addition of ionic liquids obviously enhanced the extraction yield. Experimental data showed 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIm]BF4) had the best effect.

Furthermore, a series of different length alkyl chain's ionic liquids with the same anion of BF4 were tested. Fig 1(a) showed that the length of carbon chain had great effect on extraction yield. With increasing carbon chain, the extraction rate decreased.

Therefore, [EMIm]BF4 was selected as best choice for experiment.

The concentration of [EMIm]BF4 was also studied on the extraction yield. In this study, five different concentrations (0.25 mol L^{-1}, 0.5 mol L^{-1}, 1.0 mol L^{-1}, 1.5 mol L^{-1}, and 2.0 mol L^{-1}) were selected to evaluate their effect on the extraction efficiency. Fig 1(b) showed the extraction yield increased remarkably when the [EMIm]BF4 concentrations increased from 0.25 to 0.5 mol L^{-1}. However, further increasing [EMIm]BF4 concentration lead to a slight decreasing trend, which could be attributed to the high viscosity of the ionic liquid at high concentrations which in term reduced the solution's penetration ability. 0.5 mol L^{-1} was selected as the most appropriate concentration was and used for the next experiments.

(a)

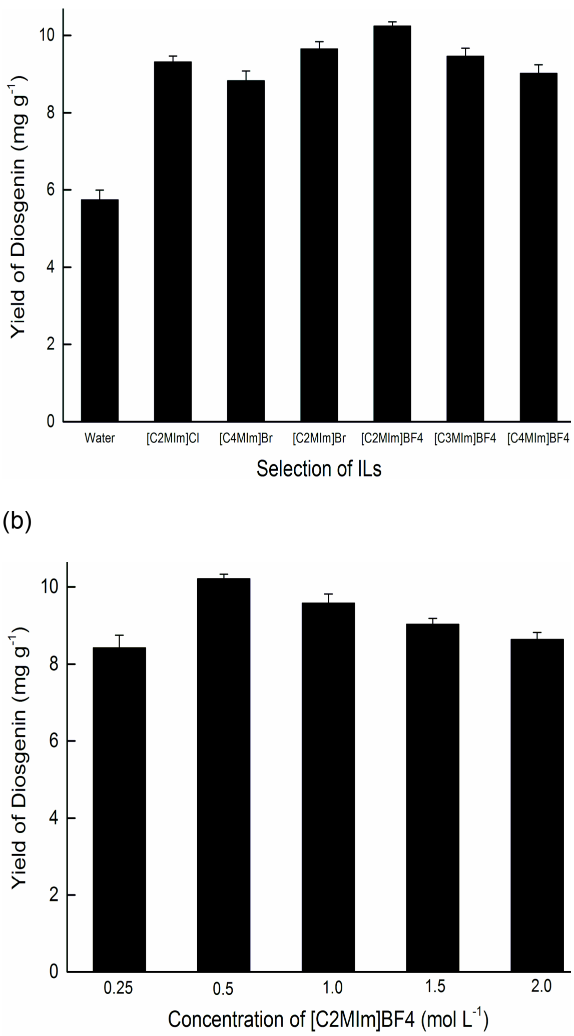

Fig 1: Selection of (a) ILs and (b) effect of $[EMIm]BF_4$ concentrations

Optimisation of IL-UMAE conditions

Three extraction parameters, liquid to solid ratio, microwave power, and extraction time, were optimized by univariate method. The Fig. 2 showed the optimum conditions: solvent to solid ratio 15:1, microwave power 500 W and extraction time 8 min. Under these optimal conditions, the highest extraction yield of diosgenin 10.24 ± 0.31 mg g^{-1} was obtained.

(a)

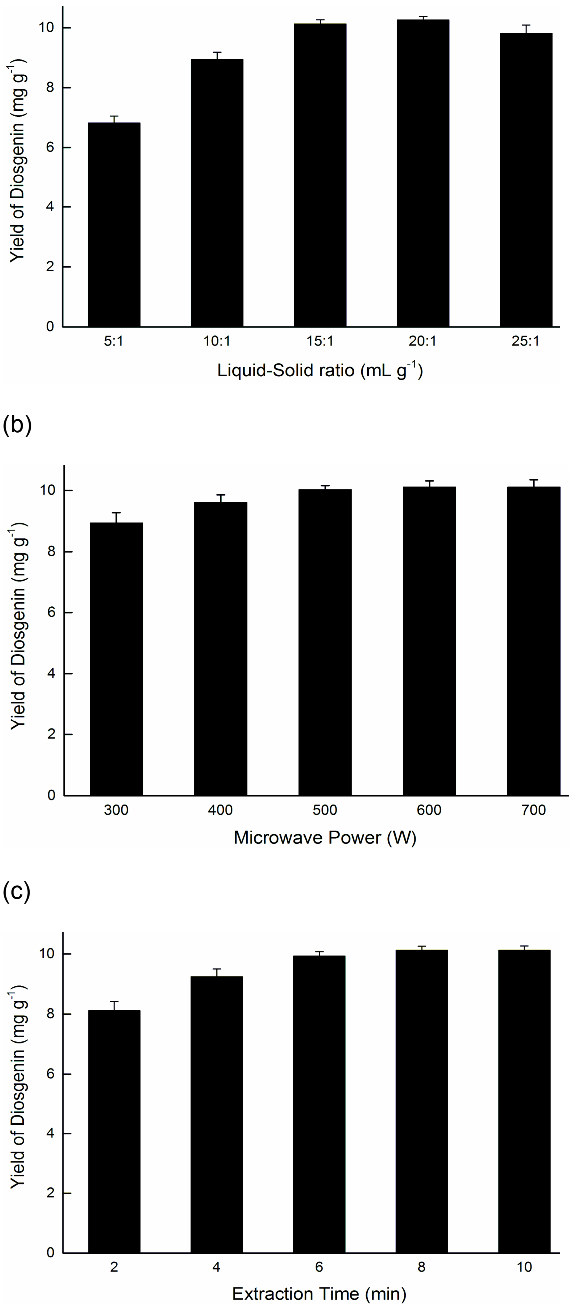

Fig 2: Optimisation of IL-UMAE conditions: (a) liquid-solid ratio, (b) microwave power, (c) extraction time

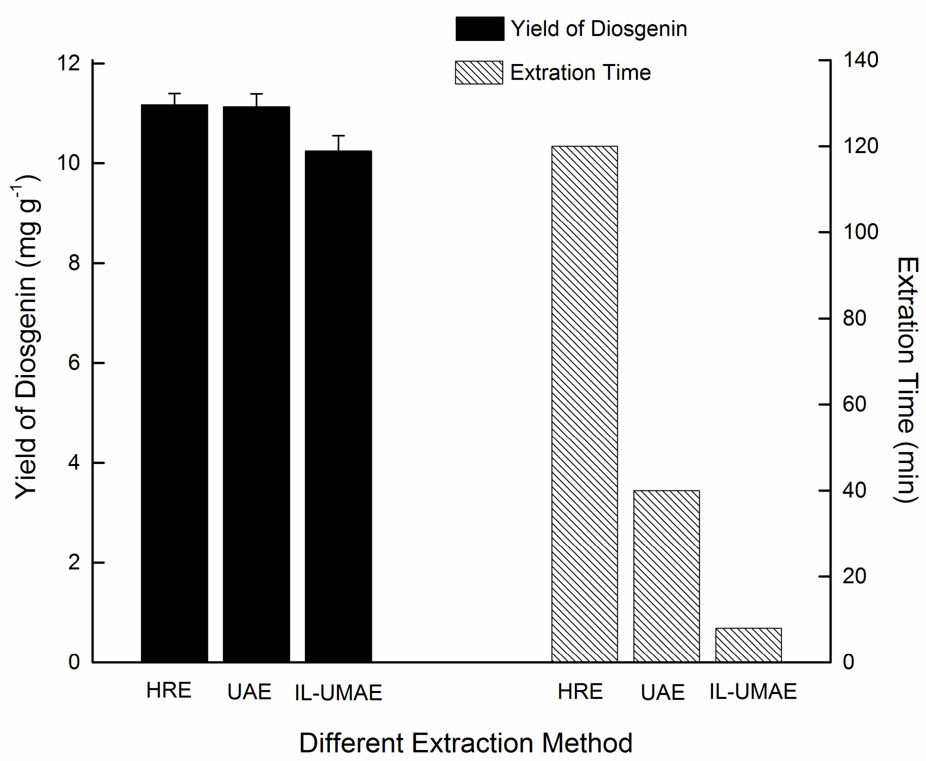

Fig 3: Comparison of heat reflux extraction (HRE) and ultrasonic assisted extraction (UAE)

Comparison of different methods

IL-UMAE under optimal conditions was compared with heat reflux extraction (HRE) and ultrasonic assisted extraction (UAE). The same sample treatment method and HPLC analysis method after extraction process were carried out. The results were showed in Fig. 3. The extraction yields of diosgenin obtained by HRE, IL-UMAE and UAE were 11.17 ± 0.23 mg g^{-1}, 10.24 ± 0.31 mg g^{-1} and 11.13 ± 0.26 mg g^{-1}, respectively.

DISCUSSION

With a variety of combinations of anions and cations, ionic liquids have significantly different physicochemical properties, which lead to distinct extraction yield [17]. Considering the hydrosoluble nature of most saponins in *Dioscorea zingiberensis* C. H. Wright rhizome, six water-soluble ILs with different anions and cations were investigated. Experimental results show that ionic liquids can enhance the extraction effect. It is probably because ILs-water solutions have much stronger interactions including π-π, ionic/charge-charge and hydrogen bonding with steroid saponins than pure water. Furthermore, extraction yield dramatically decreased when the alkyl chain length was increasing from ethyl to butyl. This phenomenon could be attributed to longer alkyl chain had greater steric hindrance effect. It also could due to increasing alkyl chain length decrease the miscibility of the ILs. Moreover, long length alkyl chain resulted in high viscosity which could prevent saponin diffusing to solvent [18].

According to the result of optimization, solvent to solid ratio, microwave power and extraction time have a larger effect on extraction yield. The solvent volume has a direct impact on extraction efficiency during the extraction process. Appropriate volume of solvent could completely submerge the materials without causing waste. In this research, five different liquid to solid ratio (5:1, 10:1, 15:1, 20:1 and 25:1) with 0.5 mol L^{-1} [EMIm]BF4 were investigated to evaluate the influence of solvent volume on the extraction yield of steroid saponins. Fig 2(a) illustrate that the extraction yield of saponins increased when the solvent to solid ratio changed clearly from 5:1 to 10:1. Then it increased slowly between 10:1 and 15:1. The difference of extraction yield was not significant from 15:1 to 20:1. However, it decreased slightly when the ratio went up to 25:1. These may be due to the interrelation between solvent volume and ultrasound energy. Appropriate volume of solvent did not affect the energy transfer, but excessive liquid to solid ratio resulted in energy absorption and dispersion

[19]. In addition, larger solvent volumes were unnecessary waste and increased the difficulty of further work. A solvent to solid ratio of 15:1 was selected for subsequent experiments.

To investigate the effect of the microwave power on the extraction efficiency, a series of comparative microwave powers (300 W, 400 W, 500 W, 600 W, and 700 W) were used out. As shown in Fig 2(b), significant differences were showed and the optimum ultrasonic power was 500 W. When power increased from 300 to 500W, the yield of the diosgenin increased distinctly. Microwave rolled in the substance was mainly through the following two ways: Polar molecules (such as H_2O) rapidly spun in the microwave electromagnetic field or rapid migration of ions in the microwave field. Unlike conventional heating method, microwave energy would directly impact on the heated material without energy loss on air and vessels. In an appropriate range of microwave power, the solvent was rapidly heated and the target substance leaching effect was strengthened. However, excessive microwave power could easily cause water bumping. Therefore, the suitable microwave power was 500W.

According to tradition, extraction yield would rise as increasing extraction time. Five kinds of extraction time (2, 4, 6, 8 and 10 min) were set to study the influence of extraction time on the yields of diosgenin. The significant effect of extraction time on the extraction yield was observed from 2 to 8 min. Fig. 2(c) indicated that the highest extraction yields sides were obtained at extraction time of 8 min. The extraction efficiency does not have significant change when time was longer than 8 min. From the perspective of saving time, 8 min was the best extraction time.

The proposal method was compared with heat reflux extraction (HRE) and ultrasonic assisted extraction (UAE). The extraction efficiency obtained by UAE was similar to that of regular HRE. The yield of diosgenin obtained by IL-UMAE method was a little less than that of others. However, IL-UMAE method greatly shortened the extraction time and still had a good extraction efficiency. It took only 8 minutes to get the 91.67 % content of steroid saponins obtained by HRE. What is more, no organic solvent was used in the IL-UMAE, which indicated that IL-UMAE was an efficient and environmentally friendly extractive technique. Considering the extraction cost and time, IL-UMAE should be the prime choice for steroid saponins extraction.

CONCLUSION

An effective ionic liquid based ultrasonic/microwave assisted extraction has been [20, 21] applied for the extraction of steroid saponins from *Dioscorea zingiberensis* C. H. Wright in an ultrasonic-microwave synergistic extraction device. The latest research shows ionic liquids provided a good extraction efficient, shorter extraction time and less expense. Considering these advantages of ionic liquids, the promissory IL-UMAE has a broad prospect in natural products extraction.

ACKNOWLEDGEMENT

This work was financially supported by the Dongguan Garden Industrial Investment Co, Ltd (Wuxi, China).

REFERENCES

1. *Wang YX, Liu H, Bao JG, Hong Y, Yang ZH, Zhang CX. The saccharification-membrane retrieval-hydrolysis (SMRH) process: a novel approach for cleaner production of diosgenin derived from Dioscorea zingiberensis. J Clean Prod 2008; 16; 1133-1137.*
2. *Peng YE, Yang ZH, Wang YX, Liu ZY, Bao JG, Hong Y. Pathways for the steroidal saponins conversion to diosgenin during acid hydrolysis of Dioscorea zingiberensis C. H. Wright. Chem Eng Res Des 2011; 89; 2620-2625.*
3. *Liu L, Dong YS, Xiu ZL. Three-liquid-phase extraction of diosgenin and steroidal saponins from fermentation of Dioscorea zingibernsis CH Wright. Process Biochem 2010; 45; 752-756.*
4. *Wu SB, Su JJ, Sun LH, Wang WX, Zhao Y, Li H, Zhang SP, Dai GH, Wang CG, Hu JF. Triterpenoids and steroids from the fruits of melia toosendan and their cytotoxic effects on two human cancer cell lines. J Nat Prod 2008; 73; 1898-1906.*
5. *Li H, Huang W, Wen YQ, Gong GH, Zhao QB, Yu G. Anti-thrombotic activity and chemical characterization of steroidal saponins from Dioscorea zingiberensis C. H. Wright. Fitoterapia 2012; 81; 1147-1156.*
6. *Wang TJ, Choi RCY, Li J, Bi CWC, Ran W, Chen XH, Dong TTX, Bi KS, Tsim KWK. Trillin, a steroidal saponin isolated from the rhizomes of Dioscorea nipponica, exerts protective effects against hyperlipidemia and oxidative stress. J Ethnopharmacol 2012; 139; 214-220.*
7. *Tong QY, He Y, Zhao QB, Qing Y, Huang W, Wu XH. Cytotoxicity and apoptosis-inducing effect of steroidal saponins from Dioscorea zingiberensis Wright against cancer cells. Steroids 2012; 77; 1219-1227.*
8. *Gong GH, Qing Y, Huang W. Anti-thrombosis effect of diosgenin extract from Dioscorea zingiberensis C.H.*

Wright in vitro and in vivo. Phytomedicine 2011; 18; 458-463.

9. Wu SB, Ji YP, Zhu JJ, Zhao Y, Xia G, Hu YH, Hu JF. Steroids from the leaves of Chinese Melia azedarach and their cytotoxic effects on human cancer cell lines. Steroids 2009; 74; 761-765.
10. Chen YY, Gu XH, Huang SQ, Li JW, Wang X, Tang J. Optimization of ultrasonic/ microwave assisted extraction (UMAE) of polysaccharides from inonotus obliquus and evaluation of its anti-tumor activities. Int J Biol Macromol 2010; 46; 429-435.
11. Zhang LF, Liu ZL. Optimization and comparison of ultrasound/microwave assisted extraction (UMAE) and ultrasonic assisted extraction (UAE) of lycopene from tomatoes. Ultrason Sonochem 2008; 15; 731-737.
12. Wu H, Guo JB, Du LM, Tian H, Hao CX, Wang ZF, Wang JY. A rapid shaking-based ionic liquid dispersive liquid phasemicro extraction for the simultaneous determination of six synthetic food colourants in soft drinks, sugar- and gelatin-based confectionery by high-performance liquid chromatography. Food Chem 2013; 141; 182-186.
13. Królikowskia M, Walczak K, Domańska U. Solvent extraction of aromatic sulfur compounds from n-heptane using the 1-ethyl-3-methylimidazolium tricyanomethanide ionic liquid. J Chem Thermodyn 2013; 65; 168-173.
14. Lovejoy KS, Davis LE, McClellan LM, Lillo LM, Welsh JD, Schmidt EN, Sanders CK, Lou AJ, Fox DT, Koppisch AT, Del Sesto RE. Evaluation of ionic liquids on phototrophic microbes and their use in biofuel extraction and isolation. J Appl Phycol 2013; 25; 973-981.
15. Wilkes JS. Properties of ionic liquid solvents for catalysis. J Mol Catal A-Chem 2004; 214; 11–17.
16. Qin Y, Wu XH, Huang W, Gong GH, Li D, He Y, Zhao YL. Acute toxicity and sub-chronic toxicity of steroidal saponins from Dioscorea zingiberensis C. H. Wright in rodents. J Ethnopharmacol 2009; 126; 543-550.
17. Ma WY, Lu YB, Hu RL, Chen JH, Zhang ZZ, Pang YJ. Application of ionic liquids based microwave-assisted extraction of three alkaloids N-nornuciferine, O-nornuciferine, and nuciferine from lotus leaf. Talanta 2013; 80; 1292-1297.
18. Bi WT, Yoon CH, ROW KH. Ultrasonic-assisted enzymatic ionic liquid-based extraction and separation of Flavonoids from chamaecyparis obtusa. J Liq Chromatogr RT 2013; 36; 2029-2043.
19. Vongsangnak W, Gua J, Chauvatcharin S, Zhongh JJ. Towards efficient extraction of notoginseng saponins from cultured cells of Panax notoginseng. Biochem Eng J 2003; 18; 115–120.
20. Lu CX, Wang HX, Lv WP, Ma CY, Xu P, Zhu J, Xie J, Liu B, Zhou QL. Ionic liquid-based ultrasonic/microwave-assisted extraction combined with UPLC for the determination of anthraquinones in Rhubarb. Chromatographia 2011; 79; 139-144.
21. Lou ZX, Wang HX, Zhu S, Zhang M, Gao Y, Ma CY, Wang ZP. Improved extraction and identification by ultra performance liquid chromatography tandem mass spectrometry of phenolic compounds in burdock leaves. J Chromatogr A 2010; 1217; 2441-2446.