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RAPID COMMUNICATION

Effect of water content of organic solvent on microwave-assisted extraction efficiency of paclitaxel from plant cell culture

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Abstract−A microwave-assisted extraction (MAE) method was used to recover the anticancer agent paclitaxel from plant cell cultures, and the extraction efficiency of the paclitaxel was determined using various organic solvents (acetone, chloroform, ethanol, methanol, and methylene chloride) and solvent concentrations. Methanol provided the highest recovery of paclitaxel (~93%) and resulted in the most severe rupturing of the biomass surface during MAE. Most of the paclitaxel (>99%) was recovered using a methanol concentration of 90% (water content: 10%), suggesting that the addition of a small amount of water improves the efficiency of MAE. Furthermore, analysis of the surface of the biomass using an electron microscope revealed that the higher the recovery of paclitaxel, the more severe the damage to the biomass surface. A comparison of the extraction efficiency between MAE and conventional solvent extraction (CSE) showed that with CSE, only up to 54% of the paclitaxel could be recovered in one extraction whereas with MAE, most of the paclitaxel (>99%) in the biomass could be recovered in one extraction.

Key words: Paclitaxel, Recovery, Microwave-assisted Extraction (MAE) Efficiency, Water Content of Solvent, Plant Cell Cultures

INTRODUCTION

Paclitaxel is a diterpenoid anticancer agent discovered in the bark of the yew tree. Its chemical composition was revealed in 1971 by Wani et al. It is one of the most effective anticancer drugs at present [1]. The anticancer mechanism of paclitaxel, which differs from those of other anticancer drugs, was revealed in 1979 by Schiff et al. to be the restriction of cancer cell division in the mitotic phase with relatively low toxicity and high activity [2,3]. Preclinical testing of paclitaxel began in 1977, followed by toxicity studies in 1980, phase I clinical testing in 1983, phase II in 1985, and phase III in 1990. It was approved by the Food and Drug Administration as a treatment for ovarian cancer in 1992, breast cancer in 1994, Kaposi's sarcoma in 1997, and non-small cell lung cancer in 1999. Paclitaxel is currently the most widely used anticancer drug [4-6]. The demand for this drug is expected to increase steadily; its indications, which currently include acute rheumatoid arthritis and Alzheimer's disease, are expanding continuously, and clinical tests for combined prescription with other treatment methods are being conducted.

The main methods of paclitaxel production include direct extraction from the yew tree, semi-synthesis involving the chemical combination of side chains after obtaining a precursor from the leaves of the yew tree, and plant cell culture from the main bioreactor after inducing callus from the yew tree and performing a seed culture [7-9]. Among these methods, plant cell culture can stably mass produce paclitaxel of consistent quality in a bioreactor without being affected by such external factors as climate and environment [5]. Most paclitaxel produced by plant cell cultures contains plant cells and debris [10]; thus it is very important to extract paclitaxel from cells efficiently. The most widely used extraction method is the recovery of paclitaxel from biomass using organic solvents. This conventional solvent extraction (CSE) method [11-13], however, requires a long extraction time and large amounts of organic solvents and has a low extraction efficiency. On the other hand, microwave-assisted extraction (MAE), which uses microwave energy and an organic solvent, can extract the target component with higher recovery and at a faster rate than CSE [14]. During MAE, microwaves heat the solvent or solvent mixture directly. In addition, direct interaction with microwaves in the presence of free water molecules in glands and vascular systems results in the subsequent rupture of plant tissue and the release of active compounds into the organic solvent, which increases extraction efficiency. Therefore, MAE is a promising alternative to conventional extraction methods, especially in the case of plant material isolation [15].

Recently, research studies have been conducted on the development of MAE methods for the extraction of saikosaponins from *Bupleurum falcatum* root [16], the anticancer drug camptothecin from *Nothapodytes foetida* [17], essential oil from cardamom [18], ginsenosides from ginseng root [19], glycyrrhizic acid from licorice root [20], and polyphenols and caffeine from green tea leaves [21]. In a previous study, we confirmed the possibility that the extraction of paclitaxel from plant cell cultures using MAE could overcome the above-mentioned problems with CSE [6]. Accordingly, the intention of the present study was to determine more specifically the extraction behavior of paclitaxel from plant cell biomass during the MAE process. First, the extraction efficiency was compared between MAE and CSE using various organic solvents (acetone, chloroform, ethanol, methanol, and methylene chloride); then the effect of solvent water content on extraction efficiency was investigated. Furthermore, the correlation between extraction efficiency (yield) and changes in the surface of biomass (plant cells) was exam-

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ined with an electron microscope.

MATERIALS AND METHODS

1. Plant Materials and Culture Conditions

A suspension of cells originating from *Taxus chinensis* was maintained in darkness at 24.0ºC with shaking at 150 rpm. The cells were cultured in modified Gamborg's B5 medium supplemented with 30 g/L sucrose, 10 mM naphthalene acetic acid, 0.2 mM 6-benzylaminopurine, 1 g/L casein hydrolysate, and 1 g/L 2-(N-morpholino) ethanesulfonic acid. Cell cultures were transferred to fresh medium every 2 weeks. During prolonged culture for production purposes, 4 mM $AgNO_3$ was added at the initiation of culture as an elicitor, and 1 and 2% (w/v) maltose were added to the medium on days 7 and 21, respectively [22]. Following cultivation, biomass was recovered by using a decanter (CA150 Clarifying Decanter; Westfalia, Germany) and a high-speed centrifuge (BTPX 205GD-35CDEFP; Alfa Laval, Sweden). The biomass was provided by Samyang Genex Company, South Korea.

2. Paclitaxel Analysis

Dried residue was redissolved in methanol for quantitative analysis with an HPLC system (SCL-10AVP; Shimadzu, Japan) with a Capcell Pak C18 column (250 mm×4.6 mm; Shiseido, Japan). Elution was performed in a gradient using a distilled water-acetonitrile mixture varying from 65 : 35 to 35 : 65 within 40 min (flow rate=1.0 mL/min). The injection volume was 20 μL, and the effluent was monitored at 227 nm with a UV detector [5]. Authentic paclitaxel (purity: 97%) was purchased from Sigma-Aldrich and used as a standard.

3. Microwave-assisted Extraction (MAE)

The microwave facility (2,450 MHz Model 1501; Korea Microwave Instrument Co., Korea) used for MAE consisted of a microwave generator, cooling system, and extraction unit (Fig. 1). A thermocouple was installed to measure temperature changes continuously during extraction. The cooling system condensed vaporized solvent, thereby returning it to the reactor. The microwave power supply (100 W) was operated by a computer program to control the temperature. According to the result of our previous study, extraction of paclitaxel using microwaves could recover the most paclitaxel from biomass with one extraction for 6 min at 40 ºC [6]. Therefore, these extraction conditions were used in the present study. Furthermore, since the mixing ratio of biomass-to-methanol (1 : 1-1 : 6, w/v) showed almost no influence on extraction efficiency, a mixing ratio of 1 : 3 (w/v) was used for experiments, considering the smooth mixing of biomass and organic solvents [11]. After extraction, the paclitaxel filtrate was recovered by vacuum filtration with filter paper (150 mm; Whatman). The filtrate was concentrated in a rotary evaporator (CCA-1100; EYELA, Japan) and dried for 24 h in a vacuum oven (UP-2000; EYELA).

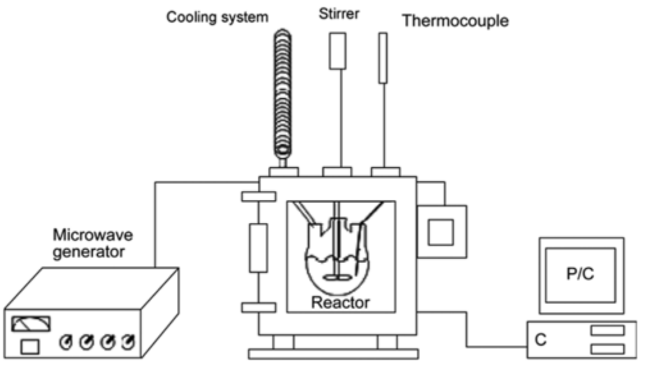

Fig. 1. Schematic diagram of the microwave-assisted extraction (MAE) process [15].

4. Conventional Solvent Extraction (CSE)

Since the highest recovery of paclitaxel could be obtained by CSE with the smallest volume of methanol compared to other solvents, it was selected as the extraction solvent in a previous study [11]. Paclitaxel was extracted with agitation for 6 min at 40 ºC using a thermostat. Other extraction conditions were identical to those of MAE to compare the efficiency and behavior of the two methods. The paclitaxel yield was defined as follows:

Yield (%)=(quantity of paclitaxel in extract/
quantity of paclitaxel in raw material)×100%

5. Electron Microscopy Analysis

After extraction, the paclitaxel filtrate was recovered by vacuum filtration and the remaining biomass was dried under vacuum for 24 h. The biomass surface was observed during the extraction process with an electron microscope (SV-35 Video Microscope System, Some Tech., Korea) at high magnification (500×). The shape of biomass surfaces in dynamic images was verified with IT-Plus software (Some Tech, Korea).

RESULTS AND DISCUSSION

1. Effect of Solvent Used for MAE

To examine variations in extraction efficiency according to the solvent used, MAE was performed with acetone, chloroform, ethanol, methanol, and methylene chloride. As shown in Fig. 2, almost no paclitaxel was recovered with methylene chloride and chloroform, whereas the highest recovery (~93%) was obtained with methanol. Thus, methylene chloride and chloroform were determined to be inappropriate extraction solvents and were excluded from further study.

The dielectric constant (ε') and dielectric loss factor (ε'') of the extraction solvent influence the heating rate of microwaves, which

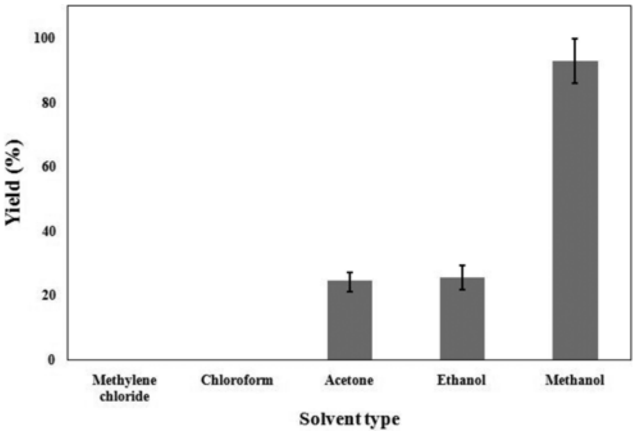

Fig. 2. Effect of various solvents on the yield of paclitaxel by MAE.

Table 1. Dielectric constants and dissipation factors of selected solvents [24]

Solvent	ε'^{a}(F/m)	ε''^{b}	tanδ^{b}
Acetone	20.7	11.5	0.5555
Methanol	32.6	15.2	0.6400
Ethanol	24.3	6.1	0.2286
Water	78.9	12	0.1500

[a] Determined at 20 °C

[b] At 2,450 MHz

plays an important role in extraction efficiency. The dielectric constant (ε') describes the polarizability of a molecule in an electric field, which indicates its ability to store electromagnetic radiation. The dielectric loss factor (ε'') measures the efficiency with which the absorbed microwave energy can be converted into heat inside a material when an electric field is applied. From these two properties can be defined another solvent dielectric property, called the dissipation factor (δ), which is expressed mathematically by:

$$\delta=\varepsilon''/\varepsilon' \tag{1}$$

This property describes the ability of the solvent to absorb microwave energy and dissipate it in the form of heat. Thus, a solvent that heats up rapidly under microwave radiation typically has a high dielectric constant and dielectric loss constant [23]. The dielectric constant, dielectric loss factor, and dissipation factor of the extraction solvents used in this study (methanol, ethanol, and acetone) were determined by Eq. (1), and the values are shown in Table 1 [24]. Methanol has a higher dielectric constant and dissipation factor than acetone and ethanol, indicating that this solvent can absorb much more microwave energy and can convert it to heat more efficiently than the other solvents. Therefore, it is estimated that the microwave heating rate of methanol was the highest and thus could provide the highest extraction efficiency.

2. Effect of Aqueous Solvent Concentration on the Efficiency of MAE

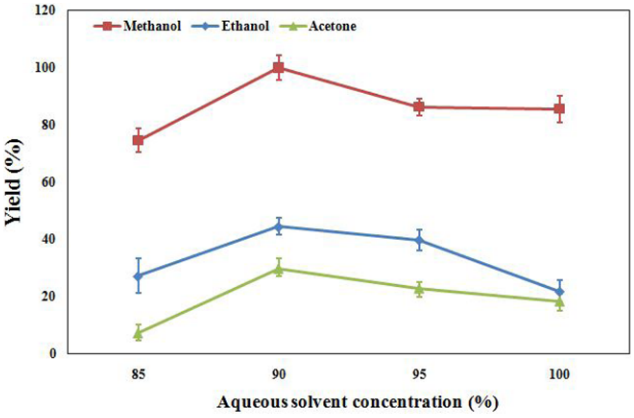

Fig. 3. Effect of extraction solvent and solvent concentration on paclitaxel yield.

The polarity index of an organic solvent plays a very important role in MAE because a polar solvent that has a higher dielectric constant can absorb more microwave energy [25]. Thus, the effects of solvent water content were investigated by conducting MAE with each organic solvent at a concentration of 85, 90, 95, and 100%. As shown in Fig. 3, the extraction efficiency was the highest over the entire concentration range when methanol was used as the solvent for MAE compared to ethanol or acetone. A previously reported CSE method [26] gave the highest recovery with the smallest volume when methanol was used for paclitaxel extraction. Hence, methanol was found to be the best extraction solvent for both MAE and CSE. Furthermore, when the efficiency of MAE was examined using an organic solvent (methanol, ethanol, or acetone) of differing water content, the paclitaxel yield was the highest at a concentration of 90% (water content: 10%). This result suggests that the water, itself a polar solvent, improved the efficiency of MAE, apparently by efficiently absorbing microwave energy and heating up. Thus, the addition of a small amount of water may have improved paclitaxel extraction efficiency by swelling the plant material and increasing the contact surface area between the plant matrix and the solvent [27]. Another reasonable explanation could be provided by the change in relative polarity of paclitaxel. The values for the polarity index of a mixture of two solvents, P_m, can be calculated from the following equation [23]:

$$P_m=\varphi_1P_1+\varphi_2P_2 \tag{2}$$

where φ_1 and φ_2 are the volume fractions of solvents 1 and 2, respectively, and P_1 and P_2 are the polarity indices of solvents 1 and 2, respectively. As can be seen in Table 2, the calculated polarity indices of the organic solvents (methanol, ethanol and acetone) including water [28] indicate that the higher the water content, the higher the polarity of the mixtures. Thus, the results in Fig. 3 show that the extraction yield increased with increasing polarity of the solvent until a solvent concentration of 90%, or a water content of 10%, was reached. Furthermore, as the water content increases, the dielectric constant of the mixture also increases, thereby enhancing the absorption of microwave energy and improving extraction efficiency. The dielectric constant of a mixed solvent, ε'_m, can generally be calculated from the following equation:

$$\varepsilon'^{1/3}_m=\Sigma(\varphi_i)\varepsilon'^{1/3}_i \tag{3}$$

where φ_i is the volume fraction of i solvent and ε'_i is the dielectric constant of i solvent [25]. As the water content increases, the dielectric constant of various mixture compositions increases but the dissipation factor (δ) decreases as a result. Compared to pure solvent, solvent mixtures can absorb higher microwave energy but can-

Table 2. Polarity indices and dielectric constants of solvent mixtures [23]

Percent solvent (%)	Polarity index of methanol and acetone[a]	Polarity index of ethanol[a]	$\varepsilon'_{Methanol}{}^{b}$	$\varepsilon'_{Ethanol}{}^{b}$	$\varepsilon'_{Acetone}{}^{b}$
100	5.100	5.20	32.6	24.3	20.7
95	5.295	5.39	34.3	26.1	22.5
90	5.490	5.58	36.1	28.0	24.4
85	5.685	5.77	37.9	30.0	26.4

[a] Calculated from Eq. (2)

[b] Calculated from Eq. (3), using values for ε from Table 1

not efficiently convert heat [23]. Therefore, it was found that MAE of paclitaxel from biomass using extraction solvents containing a small amount of water was the most effective. If the water content was too high, however, the recovery decreased, a result which is in agreement with those of the studies by Hemwimon et al. [23] and Xiao et al. [27]. Thus, recovery can be increased by using an extraction solvent of appropriate water content, but if the water content is higher than the optimal mixing composition of water and the organic solvent, it could interfere with the contact between the plant matrix and the solvent, thereby lowering the solubility of the target substance. The paclitaxel yield was the highest with the mixed composition of water and solvent when the concentration of the solvent (methanol, ethanol, or acetone) was 90% (water content: 10%), but when water content was higher, the extraction efficiency decreased. Furthermore, as shown in Fig. 4, when the biomass surface was observed with an electron microscope after MAE using organic solvents of differing water content, a correlation was found between the paclitaxel yield and the change in biomass surface: the higher the recovery from biomass, the greater the damage to the biomass surface. The reason for this observation appears to be that microwave energy affects the structure of plant cells by suddenly raising their temperature and increasing internal pressure; during the rupture process, chemical substances are rapidly exuded from cells in contact with the solvent [14,28].

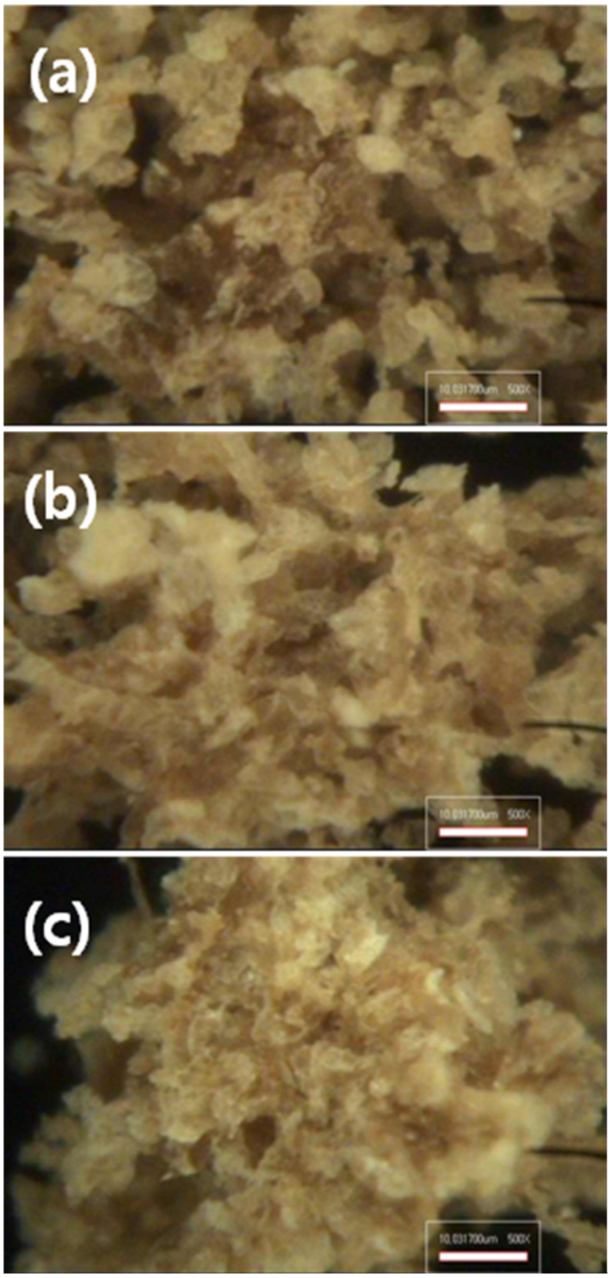

Fig. 4. Electron micrograph of biomass surface after extraction with methanol (a), ethanol (b) or acetone (c) at a concentration of 90%.

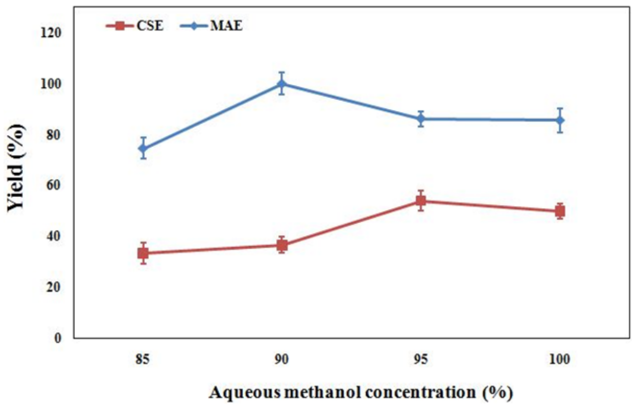

Fig. 5. Comparison of MAE and CSE at various methanol concentrations.

3. Comparison of MAE with CSE

When paclitaxel was extracted from biomass by the microwave-assisted method, the highest efficiency was obtained when methanol containing a small amount of water was used as the solvent. Therefore, the efficiencies of MAE and CSE were compared by extracting paclitaxel at a methanol concentration of 85, 90, 95 or 100%. As shown in Fig. 5, the efficiency of MAE was higher than that of CSE over the entire range of methanol concentrations. Furthermore, the paclitaxel yield varied according to the methanol water content. In the case of MAE, most of the paclitaxel in the biomass (>99%) could be recovered with only one extraction using 90% methanol. In the case of CSE, on the other hand, the extraction efficiency (yield) was nearly unaffected at a methanol concentration of 95% or higher; approximately 54% of the paclitaxel could be recovered in one extraction using 95% methanol. Therefore, it was confirmed that MAE of paclitaxel from plant cell cultures is more effective than CSE and that the extraction efficiency is improved when the solvent contains a small amount of water.

CONCLUSIONS

The extraction efficiency of the anticancer agent paclitaxel was specifically examined using MAE. This extraction process offers higher efficiency, shorter processing time, and less energy consumption than CSE for the efficient recovery of paclitaxel from biomass obtained from plant cell cultures. Various organic solvents (acetone, chloroform, ethanol, methanol, and methylene chloride) were used at a mixing ratio of biomass-to-organic solvent of 1 : 3 (w/v) to perform one extraction for 6 min at 40 °C. When methylene chloride and chloroform were used, almost no paclitaxel was extracted from the biomass. The highest recovery (~93%) was obtained when methanol was used. The heating rate of microwaves is determined by the dielectric constant (ε') and dielectric loss factor (ε''); the use of methanol in MAE resulted in a higher efficiency, a result that seems to be due to the higher heating rate of this solvent compared to the other solvents. Furthermore, the effect of the water content of the solvents methanol, ethanol, and acetone was investigated. The highest recovery of paclitaxel was obtained at a solvent concentration of 90% (water content: 10%), suggesting that the addition of a small amount of water improves the efficiency of MAE. In particular, a methanol concentration of 90% was found to be the most effective because most of the paclitaxel (>99%) was recovered under this condition. The reason for this phenomenon seems to be that the contact area between the plant matrix and the solvent is increased due to swelling of the plant material by water in MAE. If the water content is too high, however, it interferes with this contact, which may explain the decrease in paclitaxel recovery when the water content was higher than 10%. An analysis of the biomass surface using an electron microscope revealed that the higher the recovery of paclitaxel, the more severe the rupturing of the biomass surface, indicating a correlation between these two factors. In the case of CSE, a methanol concentration of 95% or higher had almost no effect on extraction efficiency, and the extraction efficiency of CSE was lower than that of MAE over the entire range of methanol concentrations tested (85-100%). In conclusion, it was found that MAE resulted in more effective recovery of paclitaxel compared to CSE.

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REFERENCES

1. M. C. Wani, H. L. Taylor, M. E. Wall, P. Coggon and A. T. McPhail, *J. Am. Chem. Soc.*, **93**, 2325 (1971).
2. P. B. Schiff, J. Fant and S. B. Horwitz, *Nature*, **277**, 665 (1979).
3. E. K. Rowinsky, L. A. Cazenave and R. C. Donehower, *J. Natl. Cancer Inst.*, **82**, 1247 (1990).
4. J. H. Kim, *Korean J. Biotechnol. Bioeng.*, **21**, 1 (2006).
5. K. Y. Jeon and J. H. Kim, *Korean J. Biotechnol. Bioeng.*, **23**, 557 (2008).
6. J. E. Hyun and J. H. Kim, *Korean J. Biotechnol. Bioeng.*, **23**, 281 (2008).
7. K. V. Rao, J. B. Hanuman, C. Alvarez, M. Stoy, J. Juchum, R. M. Davies and R. Baxley, *Pharm. Res.*, **12**, 1003 (1995).
8. E. Baloglu and D. G. Kingston, *J. Nat. Prod.*, **62**, 1068 (1999).
9. H. K. Choi, Y. S. Park, J. S. Son, S. S. Hong, J. Y. Song and G. H. Na, *Kor. J. Plant Biotechnol.*, **29**, 59 (2002).
10. J. H. Kim, C. B. Lim, I. S. Kang, S. S. Hong and H. S. Lee, *Korean J. Biotechnol. Bioeng.*, **15**, 337 (2000).
11. J. H. Kim and S. S. Hong, *Korean J. Biotechnol. Bioeng.*, **15**, 346 (2000).
12. J. H. Kim, I. S. Kang, H. K. Choi, S. S. Hong and H. S. Lee, *Process Biochem.*, **37**, 679 (2002).
13. S. H. Pyo, H. B. Park, B. K. Song, B. H. Han and J. H. Kim, *Process Biochem.*, **39**, 1985 (2004).
14. B. Zhang, R. Yang and C. Z. Liu, *Sep. Purif. Technol.*, **62**, 480 (2008).
15. W. K. Kim, H. J. Chae and J. H. Kim, *Biotechnol. Bioprocess. Eng.*, **15**, 481 (2010).
16. J. H. Kwon, Y. H. Choi, H. W. Chung and G. D. Lee, *Int. J. Food Sci. Technol.*, **41**, 67 (2005).
17. D. P. Fulzele and R. K. Satdive, *J. Chromatogr. A*, **1063**, 9 (2005).
18. M. E. Lucchesi, J. Smadja, S. Bradshaw, W. Louw and F. Chemat, *J. Food Eng.*, **79**, 1079 (2007).
19. Y. S. Youn, Y. K. Ming and S. C. Yuan, *Microchem. J.*, **74**, 131 (2003).
20. X. Pan, H. Liu, G. Jia and Y. S. Youn, *Biochem. Eng. J.*, **5**, 173 (2000).
21. X. Pan, G. Niu and H. Liu, *Chem. Eng. Proc.*, **42**, 129 (2003).
22. H. K. Choi, T. L. Adams, R. W. Stahlhut, S. I. Kim, J. H. Yun, B. K. Song, J. H. Kim, S. S. Hong and H. S. Lee, US Patent 5,871,979 (1999).
23. S. Hemwimon, P. Pavasant and A. Shotipruk, *Sep. Purif. Technol.*, **54**, 44 (2007).
24. A. Zlotorzynski, *Crit. Rev. Anal. Chem.*, **25**, 43 (1995).
25. J. Y. Hao, W. Han, S. D. Huang, B. Y. Xue and X. Deng, *Sep. Purif. Technol.*, **28**, 191 (2002).
26. S. S. Hong, B. K. Song, J. H. Kim, C. B. Lim, H. S. Lee, K. W. Kim, I. S. Kang and H. B. Park, US Patent, 5,900,979 (1999).
27. W. Xiao, L. Han and B. Shi, *Sep. Purif. Technol.*, **62**, 614 (2008).
28. H. Y. Zhou and C. Z. Liu, *J. Chromatogr. A*, **1129**, 135 (2006).