Research article

Optimization of ultrasound-assisted extraction of poly-phenols from Ajuga ciliata Bunge and evaluation of antioxidant activities in vitro

Yanfei Zhang a, Haitong Tang b, Yuchuan Zheng b, Jinzhu Li b, Le Pan b,*

a School of Information Engineering, Huangshan University, Tunxi, 245041, PR China
b School of Chemistry & Chemical Engineering, Huangshan University, Tunxi, 245041, PR China

ARTICLE INFO

Keywords:
Analytical chemistry
Food science
Ajuga ciliata Bunge
Poly-phenols
Ultrasonic
RSM
FTIR

ABSTRACT

Effective extraction of natural antioxidants from cheap plant sources is still a problem. In this paper, an excellent method of ultrasound-assisted extraction of phenolic compounds from Ajuga ciliata Bunge was studied. The effects of four factors including ethanol volume fraction, ultrasonic time, ultrasonic temperature and material liquid ratio were discussed. After single factor experiments had been investigated, a 4-factor, 3-level Box-Behnken design experiment was used to obtain the model optimum conditions, which are shown as follows: ethanol volume fraction of 41%, liquid-solid ratio of 35:1 mL/g, ultrasonic temperature of 60°C and ultrasonic time of 50 min. Under these conditions, the experimental productivity is 3.552 mg/g. The spectra of Fourier infrared and energy dispersive X-ray suggest that phenolic compounds exist in the extracts. Besides, free radical scavenging potentials of superoxide anion, hydroxyl and DPPH were measured to evaluate their antioxidant properties. This study proves that the ultrasonic-assisted extraction technique can extract phenolic compounds with antioxidant capacity from Ajuga ciliata Bunge.

1. Introduction

Biomacromolecules including nucleic acids, proteins, carbohydrates, lipids, are important parts of the organism, which not only have large molecular weight, complex structure but also have many biological functions. Once biomacromolecules are affected by abnormal factors, there will be some serious consequences. For example, oxidative damage to biological macromolecules causes many chronic diseases, such as cardiovascular disease, cancer and aging [1,2]. Some substances are called antioxidants because they have the function of resisting the oxidation of biological macromolecules and attenuating human tissue and cell damage. Natural antioxidants are a class of antioxidants that have great potential in the food, herbal, and feed industries. They can be extracted from inexpensive natural organisms, including polyphenols, flavonoids, polysaccharides and so on [3,4].

In order to effectively obtain natural antioxidants, it is important to study the extraction methods of natural organisms. Initially, the extraction of natural antioxidants was carried out by hot solvent extraction [5,6]. Subsequently, the extraction of natural antioxidants was carried out another method named Soxhlet extraction, which can overcome some shortcomings of the hot solvent extraction method. However, both two traditional methods are not efficient techniques due to its long extraction time, energy-wasting and low extract yield. Recently, some techniques have been employed to overcome these drawbacks, such as microwave-assisted extraction technique [7,8], ultrasound-assisted extraction (UAE) technique [9] and supercritical fluid extraction technique [10,11]. Among them, UAE technology for natural antioxidant extraction has great potential in the herbal and food industries [12,13], because it shows many advantages such as short extraction time, low solvent consumption and low energy consumption [14,15]. Therefore, UAE has become an emerging extraction technology of poly-phenols [16,17], flavonoids [18], and polysaccharides [19,20]. Poly-phenols are one of the most common natural antioxidants and are a class of compounds containing multiple phenolic groups in natural products that can be extracted from natural sources and studied extensively. Many researchers have studied the effective extraction of poly-phenols from natural resources and evaluation of their antioxidant activities [21,22]. It is very important to optimize the extraction factors to obtain the maximum poly-phenols content [23].

Ajuga ciliata Bunge is a perennial herb of Ajuga that is inexpensive, easy to obtain, and widely distributed in Europe, North America, China, Japan and other countries [24]. The plants of Ajuga ciliata Bunge can be
used as medicinal products in China for hundreds of years [25–27] because it has good effects on treating some symptoms of upper respiratory tract infection, tonsillitis, bronchus, high blood pressure, burns, scalds and so on [27,28]. Many researchers believed that the plants of \textit{Ajuga ciliata Bunge} have many valuable constituents, including edectosterone, cysterone, ajugasterone B, ajugasterone C, ajugalactone, saponins, alkaloids, and poly-phenols, and these constituents show various biological activities [29]. Among these biological components, poly-phenols can prevent these chronic diseases and attract the attention of many scientists.

In this paper, the UAE method was used to extract poly-phenols from \textit{Ajuga ciliata Bunge}. To obtain the best extraction conditions of UAE, response surface methodology (RSM) was utilized. Through empirical methods, optimization of extraction process factors requires a lot of time. RSM is a very effective statistical method, which is used to optimize process variables and is widely used to optimize the extraction process parameters of poly-phenols, because it can reduce the number of trials [30,31]. To our best knowledge, there are no reports on the extraction of polyphenols from \textit{Ajuga ciliata Bunge} using UAE technology by RSM. The current work is of great value compared to previous extraction studies of \textit{Ajuga ciliata Bunge}.

Firstly, fast and energy-saving ultrasonic extraction was employed instead of traditional solvent extraction. Secondly, advanced RSM was used to replace the traditional orthogonal design to optimize the extraction process. Thirdly, the technologies of FTIR and energy dispersive X-ray (EDX) were used to characterize poly-phenols extracts. Lastly, the poly-phenols of \textit{Ajuga ciliata Bunge} (PAB) have significant hydroxyl radical scavenging ability, superoxide anion radical scavenging ability and DPPH free radical scavenging ability, which indicate PAB can be utilized as natural antioxidants. This study has certain guiding significance for the extraction and application of poly-phenols from \textit{Ajuga ciliata Bunge}.

2. Materials and methods

2.1. Materials and reagents

\textit{Ajuga ciliata Bunge} was purchased from the local market in Huangshan City. After being dried for 80 h at 41 °C in a hot air oven, the dried \textit{Ajuga ciliata Bunge} was ground and conceded through a 100-mesh sieve, sealed in plastic bottle, and stored in the refrigerator at 4 °C. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Tris (hydroxymethyl)methyl aminomethane (THAM), ascorbic acid (Vc), salicylic acid, gallic acid, phenanthroline, CH$_3$CH$_2$OH, ethanol, FeSO$_4$, Na$_2$HPO$_4$, NaH$_2$PO$_4$, Li$_2$SO$_4$, (THAM), ascorbic acid (Vc), salicylic acid (SAA), Gallic acid, phenanthroline, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Tris (hydroxymethyl)methyl aminomethane (THAM), ascorbic acid (Vc), salicylic acid, gallic acid, phenanthroline, ascorbic acid (Vc), salicylic acid (SAA), Gallic acid, phenanthroline, Ciocalteu reagent was prepared by us in our laboratory.

2.2. UAE of poly-phenols from \textit{Ajuga ciliata Bunge}

The poly-phenols from \textit{Ajuga ciliata Bunge} by UAE was performed in a KQ-500E ultrasonic bath (Yuhua, China), a rectangular container with transducers at a frequency of 28 kHz. In brief, 0.2000 g of the dried ground \textit{Ajuga ciliata Bunge} plant was thoroughly mixed with 7 mL 40% ethanol and placed in a 25 mL glass tube. When the bottle was immersed in the ultrasonic bath, the liquid level in the glass tube was slightly lower than that in the ultrasonic cleaner to take advantage of the maximum ultrasonic energy. The ultrasonic power was fixed to low power of 90 W and the influence of other factors on the extraction rate of PAB were studied by single-factor design. Single-factor experiments were carried out in a designed liquid-solid (LS) ratio, ethanol volume concentration (EVC), ultrasonic temperature (UTE) and ultrasonic time (UTi) to extract poly-phenols. After the extraction tests, the ultrasonically extracted slurry was filtered with medium speed quantitative filter paper, and then filter liquor was transferred to centrifugal pipe and centrifuged at 4000 rpm for 5 min, the supernatant obtained was collected as poly-phenol of \textit{Ajuga ciliata Bunge} (PAB) in a volume bottle.

2.3. Determination of PAB content

Content of PAB was measured using Folin–Ciocalteu reagent method [32]. The Folin–Ciocalteu reagent was prepared by us in our laboratory. The main determination steps of PAB content were as follows: 0.1 mL PAB was mixed with 0.5 mL Folin–Ciocalteu reagent and 8.4 mL water. After incubated at 25 °C for 6 min, 1.0 mL 20wt% Na$_2$CO$_3$ was added and the absorbance was measured at 760 nm.

2.4. Characteristics of PAB

The supernatant was poured into a rotary evaporative bottle, and ethanol was recovered by rotary evaporation at 45 °C under vacuum. The concentrated solution was dried to constant weight at 50 °C to obtain PAB. The functional groups of PAB were characterized by Fourier transform infrared spectroscopy using a FITR-850 spectrophotometer (Gangdong, China) with KBr technique. The surface morphologies of PAB were observed by a scanning electron microscope (SEM) (S–3400N, Japan) equipped with EDX.

2.5. Statistical analysis [33,34]

Each single-factor experiment was carried out three times, the optimum technological conditions for extracting poly-phenols from \textit{Ajuga ciliata Bunge} were established by RSM [35,36] and the results of PAB were statistically analyzed by Design-Expert Software.

2.6. Antioxidant activity

2.6.1. Hydroxyl radical (HO$^•$) scavenging capacity [37,38]

Firstly, 2.0 mL of each PAB was mixed with 2.0 mL 6 mmol/L FeSO$_4$, 2.0 mL 6 mmol/L SAA-ethanol solution, 2 mL 30% H$_2$O$_2$. Then, the solution mixture was adjusted to 12 mL and incubated at 25 °C for 30 min in dark. Finally, the absorbance was read at 510 nm and designated as $A_1$. Another group of solution was measured using 2 mL water in place of 2 mL H$_2$O$_2$. The absorbance was specified as $A_{10}$. Additionally, the absorbance of the background solution was designated as $A_0$. The formula for HO$^•$ scavenging capacity of PAB is as follows:

$$ \text{HO}^\bullet \text{scavenging activity} (\%) = \frac{A_0 - (A_{10} - A_1)}{A_0} \times 100\% \quad (1) $$

2.6.2. Superoxide anion radical (O$_2^-$) scavenging activity [39]

1.5 mL of each PAB was mixed with 1.8 mL THAM-HCl (50 mmol/L, pH 8.2) and 1.0 mL pyrogallic acid (1 mmol/L). Then, they were incubated with 25 °C for 12 min and their absorbencies at 320 nm were denoted as $A_2$. The absorbance of the background solution was expressed as $A_{20}$. The formula is as follows:

$$ \text{O}_2^- \text{scavenging activity} (\%) = \frac{A_0 - A_{20} - A_2}{A_0} \times 100\% \quad (2) $$

2.6.3. DPPH radical scavenging capacity [40,41]

5.0 mL of each PAB was mixed with 1.0 mL 0.2 mmol/L DPPH and incubated in the dark at 25 °C for 30 min. The absorbance was read at 510 nm by UV-visible spectroscopy and designated as $A_3$. The absorbance of the background solution was denoted as $A_4$. The absorbance of PAB solution without added DPPH solution was read at 510 nm designated as $A_{30}$. The formula is as follows:

$$ \text{DPPH} \text{scavenging capacity} (\%) = \left(1 - \frac{A_0 - A_{30}}{A_3} \right) \times 100\% \quad (3) $$
3. Results and discussions

3.1. Single-factor experiments

Extraction experiments of PAB are carried out at various LS ratio, EVC, UTe, and UTi. The results are shown in Fig. 1, the optimal conditions for each single-factor can be observed as follows: EVC of 40%, LS ratio of 35:1 mL/g, UTi of 50 min and UTe of 50 °C. Depending on the optimal conditions of each single factor, the response values (LS ratios, X1; EVC, X2; UTe, X3 and UTi, X4) for yields of PAB is shown in Table 1.

3.2. Fitting the model

Depending on the selected factors and levels are shown in Table 1, 29 experiments of poly-phenols extraction from *Ajuga ciliata Bunge* are performed and the obtained results are listed in Table 2. Box-Behnken design (BBD) is employed to estimate the effect of response values (LS ratios, X1; EVC, X2; UTe, X3 and UTi, X4) for yields of PAB and the results of PAB extract experiment by UAE are shown in Table 2. The resulting model in terms of code to predict the optimal point of extraction are presented below in Eq. (5)

\[
Y = 3.52 + 0.14X_1 + 0.12X_2 - 0.11X_1X_2 + 0.021X_1 + 0.047X_2 + 0.14X_1X_2 + 0.075X_1X_2 + 0.20X_1X_2 + 0.088X_1X_2 + 0.22X_1X_2 - 0.95X_1^2 - 1.06X_2^2 - 0.91X_1^2 - 0.87X_2^2
\]

ANOVA is used to evaluate the significance of the model and the results are presented in Table 3. The p-values of three linear terms (LS ratios, X1; EVC, X2; UTe, X3) all less than 0.05 (marked as "*" in Table 3) indicating that the extraction yield of PAB is significantly affected by these linear terms. The p-values of two interactive terms (X1X2, X1X3) are all less than 0.05 and the extraction yield of PAB is significantly affected by X1X2 and X1X3. The p-values of other interactive terms (X1X2, X1X3, X1X4, and X2X4) are more than 0.1, which indicates that these four interaction factors are not significant. The p-values of four quadratic terms (LS ratios, EVC, UTe, and UTi) are lower than 0.0001 (marked as "**" in Table 3), which suggests that the extraction yield of PAB is extremely significantly affected by those quadratic terms. The p-value of lack-of-fit is 0.0003, which shows that the applicability of the model can accurately predict variation.

The value of R² ranges from 0 to 1. The greater the R² value is, the more accurate the model is. When the R² value is 1, the model is most accurate. According to Table 3, the value of R² is high on 0.9762, which indicates the model is very accurate. R² is influenced by the number of independent variables. To delete the influence of independent variables, Adj-R² replaced R². Adj-R² is a modification of R², it can help people better judge the pros and cons of the model [42]. The value of Adj-R² is 0.9525, which indicates that the model is very accurate. The low C.V. % (8.57%) denotes that the experiments performed are reliable. These results report that the model could work well to predict the extraction effect of *Ajuga ciliata Bunge* extraction effectiveness.

3.3. Analysis of response surfaces

The 3D response surface is presented in Fig. 2. As shown in Fig. 2A, while keeping UTe at 50 °C and UTi at 50 min, the 3D response is generated as a function of LS ratio (30:1–40:1 mL/mg) and EVC (30–50%). It can be found that EVC has a strong effect on the yield of PAB, while the extraction time has only a limited effect. From Fig. 2B, when EVC and UTi are fixed, the PAB yield raises with increment of LS ratio and UTe. From Fig. 2C, when EVC and UTi are fixed, the effect of UTi on the response variables is greater than that of LS ratio. As present in

| Table 1 | Selected factors and levels. |
|---------|-----------------------------|
| Factors | Symbol | Levels |
| LS ratio/(mL/g) | X₁ | -1 (30:1) | 0 (35:1) | 1 (40:1) |
| EVC/% | X₂ | -1 (30) | 0 (40) | 1 (50) |
| UTe/°C | X₃ | -1 (40) | 0 (50) | 1 (60) |
| UTi/min | X₄ | -1 (40) | 0 (50) | 1 (60) |

Fig. 1. The average effect of the EVC(A), LS ratio(B), UTe(C) and UTi(D) (n = 5).
Table 2
BBD and the response values for yields of PAB.

| No | X1/(mL/g) | X2/% | X3/C | X4/min | Y/(mg/g) |
|----|-----------|------|------|--------|----------|
| 1  | 0         | 0    | -1   | -1     | 2.3499   |
| 2  | -1        | 0    | 1    | 0      | 1.2470   |
| 3  | 0         | -1   | 1    | 0      | 1.0408   |
| 4  | 0         | 1    | -1   | 0      | 1.5864   |
| 5  | -1        | 0    | -1   | 0      | 1.5718   |
| 6  | 0         | 0    | 0    | -1     | 2.0697   |
| 7  | 1         | 1    | 0    | 0      | 1.8423   |
| 8  | -1        | 0    | 0    | 1      | 1.5285   |
| 9  | 0         | 0    | 0    | 0      | 3.5024   |
| 10 | 0         | 0    | 1    | 1      | 1.8156   |
| 11 | 0         | 1    | 0    | 1      | 1.7998   |
| 12 | 1         | 0    | 1    | 0      | 1.8438   |
| 13 | 0         | 0    | 0    | 0      | 3.5426   |
| 14 | 0         | 1    | 0    | 1      | 1.7087   |
| 15 | 0         | -1   | 0    | 1      | 1.2938   |
| 16 | 0         | 0    | 0    | 0      | 3.5206   |
| 17 | 1         | -1   | 0    | 1      | 1.5351   |
| 18 | 1         | 0    | 0    | -1     | 1.6427   |
| 19 | 0         | 0    | 0    | 0      | 3.4908   |
| 20 | 0         | 0    | 1    | -1     | 1.5185   |
| 21 | 1         | 1    | -1   | 0      | 1.6520   |
| 22 | -1        | 1    | 0    | 0      | 1.5630   |
| 23 | 0         | 0    | -1   | 1      | 1.4573   |
| 24 | 0         | -1   | 0    | 1      | 1.8953   |
| 25 | 0         | -1   | 0    | 1      | 1.4650   |
| 26 | 0         | 0    | 0    | 0      | 3.5206   |
| 27 | 1         | 0    | -1   | 0      | 1.5987   |
| 28 | 0         | 0    | 0    | 0      | 3.5238   |
| 29 | 0         | -1   | -1   | 0      | 1.6039   |

3.5. Antioxidant activities

3.5.1. HO•-scavenging activity

As can be seen from Fig. 3A, when the concentration was increased from 0.00 to 0.16 mg/mL, the HO•-scavenging activity of PAB increased rapidly from 0% to 83.81%. Compare to Vc, the activity of PAB is slightly stronger, indicating that poly-phenols of Ajuga ciliata Bunge has good antioxidant activity.

3.5.2. O2•-scavenging activity

As shown in Fig. 3B, as the poly-phenols concentration increased, the O2•- scavenging activity of PAB solution (15.21%–83.26%) rapidly changed. The results indicate that Vc has good O2•-scavenging activity. At low concentrations, the O2•- scavenging activity of PAB is less effective than vitamin C. However, at high concentrations, the O2•- clearance rate of PAB is superior to vitamin C. The results indicate that PAB exhibited a high O2•- scavenging activity.

3.5.3. DPPH•-scavenging activity

According to Fig. 3C, the DPPH•-scavenging activity of PAB extracted by the UAE has shown that the capacity rose with concentration. When the concentration is in the range of 0.01–0.05 mg/mL, the DPPH•-scavenging activity of PAB is slightly less than that of Vc. When the concentration was greater than 0.05 mg/mL, the antioxidant activity of PAB was the same as Vc, and the maximum inhibition percentage was 95.51%. In general, PAB exhibits significant DPPH•-scavenging activity.

According to Fig. 3C, the DPPH•-scavenging activity of PAB extracted from the UAE indicates that the capacity increases with increasing concentration. When the concentration is in the range of 0.01–0.05 mg/mL, the DPPH•-scavenging activity of PAB is slightly lower than that of Vc. When the concentration was greater than 0.05 mg/mL, the antioxidant activity of PAB was the same as that of Vc, and the maximum inhibition percentage was 95.51%. Generally, PAB exhibits significant DPPH•- scavenging activity.

3.6. Characteristics of poly-phenols

Fourier infrared spectrum can reveal characteristics of compounds extracted from Ajuga ciliata Bunge. As present in Fig. 4A, the spectrum displays many absorption bands. The peak intensity at 3400 cm⁻¹ is very strong, which provided direct evidence that a lot of hydroxyl (-OH) groups are present in PAB [43]. The peak intensity at 2924 cm⁻¹ is very weak, which provided direct evidence that a lot of hydroxyl (-OH) groups are present in PAB [43]. The peak intensity at 1450 cm⁻¹ is very strong, which provided direct evidence that a lot of hydroxyl (-OH) groups are present in PAB [43]. The peak intensity at 857 cm⁻¹ is very strong, which provided direct evidence that a lot of hydroxyl (-OH) groups are present in PAB [43]. As shown in Fig. 4B, as the poly-phenols concentration increased, the O2•- scavenging activity of PAB solution (15.21%–83.26%) rapidly changed. The results indicate that Vc has good O2•-scavenging activity. At low concentrations, the O2•- scavenging activity of PAB is less effective than vitamin C. However, at high concentrations, the O2•- clearance rate of PAB is superior to vitamin C. The results indicate that PAB exhibited a high O2•- scavenging activity.

According to Fig. 3B, when LS ratio and UTi are fixed at 0 levels, EVC pictures a strong influence on the yield of PAB, and UTi has a slight impact on the yield of PAB. According to Fig. 2E, when keeping LS ratio and UTi at 0 levels, EVC and UT are show a similar influence on the response variables. According to Fig. 2F, when LS ratio and UT are kept at 0 levels, it can be found that UTi has a strong effect on the yield of PAB, while EVC has only a limited effect. Base on 3D response surface and the model, the optimal values of the selected variables can be obtained by Expert-Design software and the optimal values are as follows: EVC of 40.54%, LS ratio of 35:1 (mL/g), UTi of 59.55 °C, UTi of 50.13 min. Under optimal conditions, the model predicted a maximum yield of $Y_{\text{max}} = 3.52531$ mg/g.
carbon (32.5wt%), followed by oxygen (10.69wt%). This is in agreement with the result of FTIR that there are a large number of oxygen-containing groups (C–O, COO–, C=O). Those results indicate that the compositions of PAB are a large number of phenolic compounds and a small amount of impurities.

4. Conclusions

In the present study, poly-phenols are effectively extracted from plants named *Ajuga ciliata Bunge* by UAE method. To get the mutual influence of factors, RSM is used to obtain the optimal conditions as follows: EVF of 41%, LS ratio of 35:1 (mL/g), UTe of 60 °C and UTi of 50 min. Under optimal conditions, the experimental maximum yield of $Y_{\text{max}} = 3.55166 \text{ mg/g}$. The spectra of Fourier infrared and EDX suggest phenolic compounds exist in the extracts. Besides, the antioxidant activity of PAB shows in the experiments of DPPH, $\cdot OH$, and $\cdot O_2$ is similar to that of Vc. It indicates that PAB using UAE method can be considered as a good natural antioxidant. This study shows that a natural antioxidant can be obtained by UAE from *Ajuga ciliata Bunge*, which may be used in pharmaceutical, food, feed, and other industries.

**Declarations**

*Author contribution statement*

Yanfei Zhang, Haitong Tan, Le Pan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Yuchuan Zheng, Jinzhu Li: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Fig. 3. The antioxidant activities of PAB: (A) HO scavenging activity, (B) O₂• scavenging activity and (C) DPPH• scavenging activity.

Fig. 4. (A) FTIR spectrum of PAB, (B) SEM images of PAB (C) EDX spectrum of PAB.

Funding statement

This work was supported by the National Natural Science Foundation of China (21401065), the Science Project of Education Department of Anhui Province (KJHS2018B04) and the Provincial Undergraduate Innovation Project (201710375012, 201810375092, 201810375112).
Table 4  
zAF intelligent quantitative results of PAB.  
| Element | Weight | Atom | Net intensity | error |
|---------|--------|------|--------------|-------|
| C K     | 32.95  | 61.94| 140.7        | 0.01  |
| N K     | 2.08   | 3.35 | 4.8          | 0.1   |
| O K     | 10.69  | 15.09| 85.7         | 0.02  |
| S K     | 0.14   | 0.1  | 3            | 0.57  |
| CuK     | 1.02   | 0.65 | 20.1         | 0.12  |
| CaK     | 53.11  | 18.87| 125.5        | 0.02  |

Competing interest statement  
The authors declare no conflict of interest.  

Additional information  
No additional information is available for this paper.

References  
[1] A. Sokol-Łotowska, J. Ozimanska, A. Wójdyło, Antioxidant activity of the phenolic compounds of hawthorn, pine and skullcap, Food Chem. 103 (2007) 853-859.
[2] J.E. Wong-Paz, D.B. Muniz-Marquez, G.C. Martinez-Avila, R.E. Belmares-Cerda, C.N. Aguilar, Ultrasound-assisted extraction of polyphenols from native plants in the Mexican desert, Ultrason. Sonochem. 22 (2015) 474-481.
[3] Q. Vuong, C. Goldsmith, T. Dang, V. Nguyen, D. Bhuyan, E. Sadeqzadeh, M. Bowyer, et al., Optimisation of ultrasound-assisted extraction conditions for phenolic content and antioxidant capacity from Euphorbia tirucalli using response surface methodology, Antioxidants 3 (2014) 604-617.
[4] D.B. Muniz-Marquez, G.C. Martinez-Avila, J.E. Wong-Paz, R. Belmares-Cerda, R. Rodriguez-Herrera, C.N. Aguilar, Ultrasound-assisted extraction of phenolic compounds from Laurus nobilis L. and their antioxidant activity, Ultrason. Sonochem. 20 (2013) 1149-1154.
[5] E.S. Ong, J.S. Cheong, D. Goh, Pressurized hot water extraction of bioactive or marker compounds in botanicals and medicinal plant materials, J. Chromatogr. A 1112 (2006) 92-102.
[6] H.N. Sin, S. Yusof, S. Nia, R.A. Rahman, Optimization of hot water extraction for sapodilla juice using response surface methodology, J. Food Eng. 74 (2006) 352-358.
[7] T. Wu, J. Yan, R. Liu, M.F. Marcone, H.A. Aina, R. Tsao, Optimization of microwave-assisted extraction of phenolics from potato and its downstream  

medium[J], Chem. Eng. J. 235 (1) (2014) 100-108.
[8] T. Belwal, P. Dhyan, I.D. Bhatt, R.S. Rawal, V. Pande, Optimization extraction conditions for improving phenolic content and antioxidant activity in Berberis asiatica fruits using response surface methodology (RSM), Food Chem. 207 (2016) 115-124.
[9] R. Dolores, S. Raquel, G.L. Adianez, Sonochemical synthesis of iron oxide nanoparticles loaded with folate and cisplatin: effect of ultrasonic frequency, Ultrason. Sonochem. 23 (2015) 391-398.
[10] Z. Zhang, X. Wang, M. Zhao, K. Qian, Optimization of polysaccharides extraction from Celmatis houchouensis Tamura and its antioxidant activity, Carbohydr. Polym. 111 (2014) 762-767.
[11] Q. Li, N. Yu, Y. Wang, Y. Sun, K. Wu, Guan, Optimization extraction of Brugniera gymnorrhiza polysaccharides with radical scavenging activities, Carbohydr. Polym. 96 (2013) 148-155.
[12] J. Lai, C. Xin, Y. Zhao, B. Feng, C. He, Y. Dong, S. Wei, et al., Optimization of ultrasound assisted extraction of antioxidants from black soybean (Glycine max var. soybean) using response surface methodology, Food Chem. 149 (2015) 153-158.
[13] A.L. Fogarasi, S. Kun, G. Tanko, E. Stefanovits-Banyai, B. Hegyesy-Vecsei, A comparative assessment of antioxidant properties, total phenolic content of einkorn, wheat, barley and their malts, Food Chem. 167 (2015) 1-6.
[14] P. Mazere, H. Josyadeh, M. Noshad, M. Hojati, Polyacrylazide of cappor (Cappori spinosa L.) Leaf: extraction optimization, antioxidant potential and antimicrobial activity, Int. J. Biol. Macrocor. 95 (2017) 224-231.
[15] M. Naushad, Surfactant assisted nano-composite cation exchanger: development, characterization and applications for the removal of toxic Pb2+ from aqueous medium[J], Chem. Eng. J. 225 (1) (2014) 100-108.
[16] M. Naushad, T. Ahamed, B.M. Al-Mansari, et al., Nickel ferrite bearing nitrogen-doped mesoporous carbon as efficient adsorbent for the removal of highly toxic metal ion from aqueous medium[J], Chem. Eng. J. 330 (2017) 1351-1360.
[17] M. Naushad, T. Ahamed, G. Sharma, et al., Synthesis and characterization of a new starch/SnO2 nanocomposite for efficient adsorption of toxic Hg2+ metal ion[J], Chem. Eng. J. 300 (2016) 306-316.
[46] R.E. Ghitescu, I. Volf, C. Carausu, A.M. Buhlmann, I.A. Gilca, V.I. Poga, Optimization of ultrasound-assisted extraction of polyphenols from spruce wood bark, Ultrason. Sonochem. 22 (2015) 535–541.

[47] A.A. Alqadami, M. Naushad, M.A. Abdalla, et al., Efficient removal of toxic metal ions from wastewater using a recyclable nanocomposite: a study of adsorption parameters and interaction mechanism (J), J. Clean. Prod. 156 (2017) 426–436.

[48] S.M. Alshehri, M. Naushad, T. Ahamad, et al., Synthesis, characterization of curcumin based ecofriendly antimicrobial bio-adsorbent for the removal of phenol from aqueous medium (J), Chem. Eng. J. 254 (7) (2014) 181–189.