Optimal extraction, purification and antioxidant activity of total flavonoid from endophytic fungi of *Conyza blinii* H. Lév

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**Background.** Flavonoid is widely used in the market because of its antibacterial, antiviral, and antioxidant activities. But the production speed of flavonoid is limited by the growth of plants. CBL9 (*Chaetomium cruentum*) is a Flavonoid-producing endophytic fungi from *Conyza blinii* H. Lév, which has potential to produce flavonoid. **Methods.** In this study, we isolated total flavonoid from endophytic fungus CBL9 of *Conyza blinii* H. Lév using macroporous resin D101. The process was optimized by response surface and the best extraction process was obtained. The antioxidant activities of total flavonoid was analyzed in vitro. **Results.** It was found that the best parameters were 25°C, pH 2.80, 1.85 h, and the adsorption ratio reached (64.14 ± 0.04)%. 60% ethanol was the best elution solvent. The elution ratio of total flavonoid reached to (81.54 ± 0.03)%, and the purity was 7.13%, which was increased by 14.55 times compared with the original fermentation broth. Moreover its purity could rise to 13.69% after precipitated by ethanol, which is very close to 14.10% prepared by ethyl acetate extraction. In the antioxidant research, the clearance ratio of L9F-M on DPPH, ABTS, ·OH, ·O₂⁻, (96.44 ± 0.04)% and (75.33 ± 0.03)%, (73.79 ± 0.02)%, (31.14 ± 0.01)% at maximum mass concentration, was higher than L9F. **Conclusion.** The result indicated using macroporous resin in the extraction of total flavonoid from endophytic fungus is better than organic solvents with higher extraction ratio, safety and lower cost. And in vitro test indicated that the flavonoid extracted by macroporous resin have good antioxidant activity, providing more evidence for the production of flavonoid by biological fermentation method.
Optimal Extraction, Purification and Antioxidant Activity of Total
Flavonoid from Endophytic Fungi of *Conyza blinii* H. Lév

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ABSTRACT

Background. Flavonoid is widely used in the market because of its antibacterial, antiviral, and antioxidative activities. But the production speed of flavonoid is limited by the growth of plants. CBL9 (*Chaetomium cruentum*) is a Flavonoid-producing endophytic fungi from *Conyza blinii* H. Lév, which has potential to produce flavonoid.

Methods. In this study, we isolated total flavonoid from endophytic fungus CBL9 of *Conyza blinii* H. Lév using macroporous resin D101. The process was optimized by response surface and the best extraction process was obtained. The antioxidant activities of total flavonoid was analyzed in vitro.

Results. It was found that the best parameters were 25°C, pH 2.80, 1.85 h, and the adsorption
ratio reached (64.14 ± 0.04) %. 60% ethanol was the best elution solvent. The elution ratio of total flavonoid reached to (81.54 ± 0.03)%, and the purity was 7.13%, which was increased by 14.55 times compared with the original fermentation broth. Moreover its purity could rise to 13.69% after precipitated by ethanol, which is very close to 14.10% prepared by ethyl acetate extraction. In the antioxidant research, the clearance ratio of L9F-M on DPPH, ABTS, \( \cdot \text{OH} \), \( \cdot \text{O}^2^- \), (96.44 ± 0.04)% and (75.33 ± 0.03)%, (73.79 ± 0.02)%, (31.14 ± 0.01)% at maximum mass concentration, was higher than L9F.

**Conclusion.** The result indicated using macroporous resin in the extraction of total flavonoid from endophytic fungus is better than organic solvents with higher extraction ratio, safety and lower cost. And *in vitro* test indicated that the flavonoid extracted by macroporous resin have good antioxidant activity, providing more evidence for the production of flavonoid by biological fermentation method.

**Keywords:** *Chaetomium cruentum*; Flavonoid; Macroporous resin; Response surface

**INTRODUCTION**

Flavonoid is an important secondary metabolites of plants, who owns diverse pharmacological activities owning to complex structure types (Yonekura-Sakakibara *et al.*, 2019). For example, flavonoid has a strong antioxidant effect on blood circulation and cardiovascular system (Echeverría *et al.*, 2017); Calycosin have significant antiviral activity both *in vivo and in vitro* (Zhu *et al.*, 2009); Most flavonoid has a significant inhibitory effect on the growth of bacteria including *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* (Yousef *et al.*, 2007; Gadkowski *et al.*, 2019).

Endophytic fungi widely exist in advanced plant. It has obvious host specificity and tissue
specificity (Martin *et al*., 2013; Carrol *et al*., 1978). Endophytic fungi is able to produce the same or similar secondary metabolites of the host, including flavonoid with excellent activity (Qiu *et al*., 2010; Shih *et al*., 2017; Shou-Jie *et al*., 2018; Chi *et al*., 2019). CBL9 is a Flavonoid-producing endophytic fungus from *Conyza blinii* H. Lév, which belongs to *Chaetomium* and is used to produce flavonoid with excellent antioxidant effect in vitro (Tang *et al*., 2020).

Macroporous resins have been used in the extraction of flavonoid widely as a result of its advantages (Du *et al*., 2012; Li *et al*., 2012; Chen *et al*., 2013; Lihu *et al*., 2018). But macroporous resin has not been used in the endophytic fungi of *Conyza blinii* H. Lév. currently. The macroporous resin D101 was used to extract the total flavonoids of CBL9 for further uses, and the response surface method was optimized to obtain the best process to extract the total flavonoid of the endophytic fungi of *Conyza blinii* H. Lév.

**MATERIALS AND METHODS**

2.1 Plant material

The endophytic fungus CBL9 separated from *Conyza blinii* H. Lév: Biochemistry and Molecular Biology Laboratory of Sichuan Agricultural University preserved

2.2 Chemical reagents

2, 2-Diphenyl-1-picrylhydrazyl (DPPH, HPLC) was purchased from Yuanye Biotechnology Co. (Shanghai, P. R. China). Ferrous Sulfate Heptahydrate (FeSO₄•7H₂O), hydrogen peroxide (30%H₂O₂), Pyrogallol and Concentrated hydrochloric acid were purchased from Xilong Chemical Co. (Sichuan, P. R. China). Ascorbic acid and Vitamin C (Vc, AR) were purchased from Sinopharm Chemical Reagent Co. (Shanghai, P. R. China). Other chemicals and solvents used in this study were analytical grade.
2.3 Study on optimization of extraction process of crude flavonoid

2.3.1 Preparation of raw mater

The flavonoid-producing endophytic fungus CBL9 was inoculated into fresh PDA medium and cultured with shaking at 28°C until the mycelial pellets grew to a certain condition (Bacteria is not growing and the concentration of flavonoids in the fermentation broth reaches 10mg/L), then fermentation broth was rotary evaporation at 50°C and raw material (L9F) was obtained after freeze-drying.

2.3.2 Ethyl acetate extraction

The extraction was conducted by following the method of Saraswaty with slight modifications (Saraswaty et al., 2013). 200mL concentrated fermentation broth was mixed with two times the volume of ethyl acetate to extract the fermentation broth, and repeat extraction three times. Crude flavonoid (L9F-E) was obtained by concentrating by evaporation under freeze-drying.

2.3.3 Preparation of standard curve

Sodium nitrite-aluminum nitrate colorimetric method was used to draw rutin standard song by following the method of Tang (Tang et al., 2020). The standard curve equation: \( A=0.4164C-0.0003 \) \( (R^2=0.9994) \).

2.3.4 Single factor test

The single factor test method was conducted with slight modifications (Bi et al., 2012) and the Macroporous resin D101 was used (Lihu et al., 2018). First, 1.0 g wet resin pretreated was put into 15 conical flasks and mixed with certain L9F. Then the mixture was incubated at different temperature, pH for different time. The adsorption ratio of flavonoid was measured.

2.3.5 Response surface analysis

According to the single-factor study, the Box-Behnken optimization study was designed (Yu
et al., 2019) with three levels (adsorption time, temperature, and pH). The adsorption ratio was chosen as inspection index. Design-Expert was used to analyze based on Box-Behnken data. Possible mathematical model is:

\[ Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ij} x_i x_j + \sum \beta_{ij} x_i^2 \]

\( Y \) is the predicted response value; \( \beta_0, \beta_i, \beta_{ii} \) and \( \beta_{ij} \) is the regression coefficient representing the interaction of intercept, linear, squared and two factors; \( x_i \) and \( x_j \) is the independent factor of encoding \((i \neq j)\).

2.4 Influence of eluent concentration

Gradient volume fraction of ethanol-water solution was used to elute saturated adsorbent at a certain flow rate (1 mL/min). Its purity and elution ratio was measured. The total flavonoid (L9F-M) was extracted under optimal condition we got in 2.3.5 and 2.4.

2.5 In vitro antioxidant activity assay

2.5.1 DPPH radical scavenging assay

The DPPH radical scavenging activities was collected as previously described in Kaur and Tsimogiannis & Oreopoulou (Kaur et al., 2020; Tsimogiannis & Oreopoulou 2005). Vc was used as the standard antioxidant. L9F was separately dissolved in distilled water to prepare flavonoid solutions of different concentrations (0.01, 0.02, 0.03... 0.09, and 0.10 mg/mL). Equal volume of DPPH solution was mixed with different concentrations of flavonoid solution. The mixture was shaken and then put in a dark place for 30 min. Finally, the absorbance was measured at 517 nm by a spectrophotometer and each experimental group had 3 parallel controls.

\[ Y (%) = \left[ 1 - \frac{(A_1 - A_2)}{A_3} \right] \times 100\% \]
$Y(\%)$ is the DPPH radical scavenging activity $A_1$ is the absorbance of the sample with DPPH, $A_2$ is the absorbance of the sample without DPPH, and $A_3$ is the absorbance of DPPH without the sample.

2.5.2 ABTS radical scavenging assay

The ABTS radical scavenging activities was collected as previously described in Kaur and Zhang (Kaur et al., 2020; Zhang et al., 2018). Vc was used as the standard antioxidant. L9F was separately dissolved in distilled water to prepare flavonoid solutions of different concentrations (0.01, 0.02, 0.03 ... 0.09, and 0.10 mg/mL). Two milliliters of ABTS solution was mixed with one hundred Microliters of different flavonoid solution. The mixture incubated in a dark place at room temperature for 6 min. Finally, the absorbance was measured at 734 nm by a spectrophotometer and each experimental group had 3 parallel controls.

$Y(\%) = \left[ 1 - \frac{A_1 - A_2}{A_3} \right] \times 100\%$

$Y(\%)$ is the ABTS radical scavenging activity, $A_1$ is the absorbance of the sample with ABTS, $A_2$ is the absorbance of the sample without ABTS, and $A_3$ is the absorbance of ABTS without the sample.

2.5.3 Hydroxyl radical (·OH) scavenging assay

The hydroxyl radical scavenging activities of sample was collected as previously described in Chobot (Chobot et al., 2011), and Vc was used as the standard antioxidant. L9F was separately dissolved in distilled water to prepare flavonoid solutions of different concentrations (0.1, 0.2, 0.3 ... 0.9, and 1.0 mg/mL). A 0.5 mL flavonoid solutions of
each concentration was mixed with 1 mL of Salicylic acid (6 mM), 1.5 mL of phosphate
buffer solution (PBS, 0.15 M, pH 7.4), 1 mL of ferrous sulfate (6 mM) and 0.5 mL of
H$_2$O$_2$ solution (0.01%). Then, the mixtures were incubated at 37 °C for 30 min. Finally,
the absorbance was measured at 510 nm by a spectrophotometer and each experimental
group had 3 parallel controls.

\[ Y(\%) = \left[ \frac{(A_1 - A_2)}{(A_3 - A_2)} \right] \times 100\% \]

Y(%) is the •OH radical scavenging activity (%), A$_1$ is the absorbance of the sample after
reaction with hydroxyl radicals, A$_2$ is the absorbance of the sample, and A$_3$ is the absorbance
without H$_2$O$_2$.

2.5.4 Superoxide radical (•O$^2_-$) scavenging assay

The superoxide radical scavenging activities of sample was collected as previously
described in Zhishen and Leong (Zhishen et al., 1999; Leong et al., 2008). Similarly, Vc was
used as the standard antioxidant. L9F were separately dissolved in distilled water to
prepare polysaccharide solutions of different concentrations 0.01, 0.02, 0.03 ... 0.09, and
0.10 mg/mL). A 1.0 mL sample of each concentration was mixed with 3.0 mL of Tris-Hcl
buffer (pH 8.2), 0.8 mL of Pyrogallol (0.05 M). Then, the mixtures were bathed at 25°C
for 5 min and 1.0 mL HCl (8.00 M) was mixed after that. Finally, the absorbance was
measured at 325 nm using a spectrophotometer.

\[ Y(\%) = \left[ 1 - \frac{(A_1/A_2)} \right] \times 100\% \]

Y is the•O$^2_-$ radical scavenging activity (%), A$_1$ is the absorbance of the sample and A$_2$
is the absorbance of the control.

RESULTS
3.1 The result of Single factor test

3.1.1 The influence of adsorption time

Fig.1 shows that the adsorption ratio increases first and then stabilizes with the increase of adsorption time. The adsorption ratio reached to the maximum first at 1h, which was 59.06 ± 0.03%.

3.1.2 The influence of pH

Fig.2 shows that the adsorption ratio increases first, then goes down with the increase of pH. The adsorption ratio reach to the maximum at pH 3.5, which was (31.65 ± 0.03)%. There was no adsorption when pH was more than 5, which may be caused by the change of the flavonoid structure and the deactivation (Wu et al., 2017) or the weakening of the adsorption capacity of the macroporous resin (Li et al., 2007) under this pH condition.

3.1.3 The effect of temperature

Fig.3 shows that the adsorption ratio increases first and then goes down with the increase of temperature. The adsorption ratio reached to the maximum first at 30°C, which was 51.26 ± 0.02%.

3.2 Optimization of Extraction Process by Response Surface Methodology

3.2.1 Selection of analysis factor level

According to Box-Benhnken's central combination test design principle and single-factor test results, three factors (temperature, pH, and time) that have significant effects on the extraction of total flavonoid were selected, and the three-factor three-level response is adopted, which is shown in Table 1.

3.2.2 Response surface analysis experiment design scheme
A (temperature), B (pH), and C (adsorption time) was taken as independent variables, and Y (total flavonoid adsorption ratio) was taken as the response value. The test plan and results are shown in Table 2.

3.2.3 Establishment and Analysis of Multivariate Quadratic Response Surface Regression Model

Quadratic regression response surface analysis performs in Table 2, and a multiple quadratic response surface regression model was established: 

\[ Y = 0.47 - 0.056A - 0.042B + 0.031C + 0.088AB - 0.043AC + 4 \times 10^{-4}BC - 0.064A^2 - 0.047B^2 + 0.056C^2. \]

The variance analysis of each factor is shown in Table 3.

Table 3 shows that the model is significant (p<0.05) and the Prob>F value of the decisive factor coefficient such as A (temperature), B (pH), C (adsorption time), AB (interaction between temperature and pH), AC (interaction between temperature and adsorption time) are 0.0008, 0.0039, 0.0161, 0.0004, 0.0192 (p<0.05), indicating that the model has a good fit. In addition, the factors affecting the extraction ratio of total flavonoid were A (temperature), B (pH), and C (adsorption time) in order of magnitude, and the temperature reached a significant level (p<0.001).

In this experiment, the interaction between AB and AC has significant effect. The results are shown in Fig.4 and Fig.5, and the interaction between BC is shown in Fig.6. It can be seen from the response surface diagram that the extraction ratio of total flavonoid first increases and then decreases and increases in the end with the increase of A (temperature). B is in the range of -1.0 to 0. Due to the interaction of A, the extraction ratio of total flavonoid is higher. C is in the range of 0 to 1.0, due to the interaction of A, the extraction ratio of total flavonoid is higher.
3.2.4 Model verification

It is obtained that the supreme extraction ratio predicted is 64.2% under the process conditions of 25.02°C, pH 2.80 and 1.85 h by solving the inverse matrix of the quadratic polynomial mathematical model of the total flavonoid yield.

The optimal conditions were revised to 25°C, pH 2.80, and 1.85 h to extract the total flavonoid by the above to check the validity. The actual adsorption ratio was (64.14 ± 0.04)%, which is close to the theoretical value.

3.3 The effect of eluent concentration

**Fig.7** shows the ethanol elution effect with different volume fractions. As the volume fraction of the ethanol increases, the elution ratio is increasing. When 60% ethanol was used, the elution ratio of total flavonoid reached to (81.54 ± 0.03)% and the purity of flavonoid was 7.13%. When 80% ethanol was used, although the elution ratio rises to (90.49 ± 0.03)%, the purity dropped to 3.61%. Therefore 60% ethanol is selected for elution considering the purity and elution ratio.

The total flavonoid prepared by ethyl acetate extraction method, whose purity is 14.1%, but extraction ratio is only (31.68 ± 0.04)%, not only consumes a large amount of raw material and ethyl acetate, but also causes environmental problems. Certain pollution. The macroporous resin adsorption method is more economical and environmentally friendly, and the eluate’s purity can rise to 13.69% with precipitated by absolute ethanol.

3.4 *In vitro* antioxidant activity

3.4.1 DPPH radical scavenging assay

DPPH has been widely accepted as a tool for estimating the free radical scavenging activities of antioxidants (Khled Khoudja *et al.*, 2014). **Fig.8** shows that the DPPH clearance ratio goes up...
as the concentration of each sample increases and it tends to be flat when the sample mass concentration is greater than 0.05 mg/mL. When the concentration reaches the maximum (0.1 mg/mL), the clearance ratio of total flavonoid on DPPH increases from (95.33 ± 0.01)% to (96.44 ± 0.04)% after purification, and Vc’s clearance ratio is (94.18 ± 0.002)%. It can be seen that the clearance effect of flavonoid on DPPH slightly better than that before purification, and the clearance effect of flavonoid on DPPH is close to Vc.

3.4.2 ABTS radical scavenging assay

ABTS has been widely accepted as a tool for estimating the free radical scavenging activities of antioxidants (Thaipong et al., 2012). Fig.9 shows that the ABTS clearance ratio goes up as the mass concentration of each sample increases. When the concentration reaches the maximum (0.1 mg/mL), the clearance ratio of total flavonoid on ABTS increases from (74.06 ± 0.04)% to (75.33 ± 0.03)% after purification, and Vc’s clearance ratio is (71.74 ± 0.05)%. It can be seen that the clearance effect of flavonoid on ABTS slightly better than that before purification, and the clearance effect of flavonoid on ABTS is better than Vc.

3.4.3 Hydroxyl radical (·OH) scavenging assay

Hydroxyl radicals are very active that has been associated with cancer risk when it accumulated in the body excessively (Sakanaka et al., 2005). Fig.10 shows that the ·OH clearance ratio goes up as the mass concentration of each sample increases and it tends to be flat when the sample mass concentration is greater than 0.3 mg/mL, but the clearance ratio of the sample before purification shows a downward trend, which may be the presence of oxidized and discolored impurities in the original fermentation broth. When the sample concentration reaches the maximum (1 mg/mL), the clearance ratio of total flavonoid on ·OH increases from (3.98 ± 0.02)% to (73.79 ± 0.02)% after purification,
and Vc’s clearance ratio is $(93.72 \pm 0.01)\%$. It can be seen that the clearance effect of flavonoid on ·OH better than that before purification.

3.4.3 Superoxide radical ($\cdot\text{O}_2^-$) scavenging assay

Superoxide radical is known to be very harmful to cellular components as a precursor of more reactive oxygen species, contributing to tissue damage and various diseases (Ozsoy et al., 2008; Aruoma et al., 2006). Fig.11 shows that the $\cdot\text{O}_2^-$ clearance ratio goes up as the mass concentration of each sample increases and it tends to be flat when the sample mass concentration is greater than 0.05 mg/mL, but the clearance ratio of the sample before purification shows a downward trend, which may be the presence of oxidized and discolored impurities in the original fermentation broth. When the sample concentration reaches the maximum (0.1 mg/mL), the clearance ratio of total flavonoid on $\cdot\text{O}_2^-$ increases from $(0.15 \pm 0.016)\%$ to $(28.11 \pm 0.01)\%$ after purification, and Vc’s clearance ratio is $(31.14 \pm 0.01)\%$. It can be seen that the clearance effect of flavonoid on $\cdot\text{O}_2^-$ better than that before purification.

DISCUSSION

Flavonoid has attracted more and more attention because of its complex structure and function in these years (Baran et al., 2020; Zou et al., 2020; Xiao-Hui et al., 2020). However, flavonoid from plants is mainly used in the market now. The regeneration ratio is affected by the natural growth of plants, and the yield is limited. Therefore, the objective of this study was to extract flavonoid by macroporous resin and analyse antioxidant activities. It has been shown that the extraction procedure has a significant impact on the yield and structural characteristics of flavonoid, as well as their biological activities (Taghinia
The macroporous resin adsorption method has been widely used in the extraction and purification of total flavonoid from plants (Du et al., 2012; Lihu et al., 2018). The macroporous resin adsorption method is more economical and environmentally friendly with similar purity and higher extraction ratio compared with commonly used organic solvent method.

It has been found that the generation of reactive oxygen species (ROS) and the corresponding response to oxidative stress are critical factors in the outbreak of several human diseases (Lee & Lee, 2006). Antioxidants have vital functions against ROS in the biological system (Zhang et al., 2015). In the present study, the Antioxidant activity of flavonoid was studied by DPPH, ABTS, superoxide radical and hydroxyl radicals. The results showed that flavonoid exhibited stronger antioxidant activity than before against DPPH, ABTS, superoxide radical and hydroxyl radicals, and the clearance ratio is highly closed to total flavonoid isolated by ethyl acetate (Tang et al., 2020).

These results indicated that the total flavonoid extracted from the endophytic fungus L9 from Conyza blinii H. Lév by macroporous resin has good antioxidant activity, which is closed to ethyl acetate extraction. And the method with macroporous resin is better than which with Ethyl acetate in many aspects. Further scientific work in our laboratory is in progress to separate it.

**Conclusions**

In the present study, we used the response surface method to optimize the extraction of total flavonoid from the endophytic fungus L9 from Conyza blinii H. Lév by macroporous resin the first time. It was found that the best adsorption ratio reached (64.14 ± 0.04) % and the purity of the total flavonoid is increased by 14.55 times compared with the original fermentation broth. In the antioxidant research, L9F-M has good antioxidant activity in vitro. These results demonstrate
that macroporous resin in the extraction of total flavonoid from endophytic fungus is better than organic solvents with higher extraction ratio, safety, lower cost and good antioxidant, which provides more evidence for the production of flavonoid by biological fermentation method. However, the further application remain to be explored in future studies.

ADDITIONAL INFORMATION AND DECLARATIONS

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Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

• Shuheng Zhao conceived and designed the experiments, performed the experiments, and prepared figures and/or tables.

• Xulong Wu performed the experiments, analyzed the data, prepared figures and/or tables.

• Xiaoyu Duan, Caixia Zhou, Zhiqiao Zhao performed the experiments, prepared figures and/or tables.

• Zizhong Tang conceived and designed the experiments, drafted the work, approved the final work.

• Hui Chen, Yujun Wan, Yirong Xiao, Hong Chen analyzed the data, drafted the work or revised
it critically for important content.

REFERENCES

Akubugwo IE, Obasi NA, Chinyere GC, and Ugbohu AE. 2007. Nutritional and chemical value of *Amaranthus hybridus* L. leaves from Afikpo, Nigeri. *African Journal of Biotechnology* 6(24):2833-2839. DOI: 10.5897/ajb2007.000-2452

Yonekura-Sakikibara K, Higashi Y, Nakabayashi R. 2019. The Origin and Evolution of Plant Flavonoid Metabolism. *Other* 10:943. DOI: 10.3389/fpls.2019.00943

Echeverría Javier, Julia O, Leonora M, Urzúa Alejandro, Marcela W.. 2017. Structure-Activity and Lipophilicity Relationships of Selected Antibacterial Natural Flavones and Flavanones of Chilean Flora. *Molecules* 22(4):608. DOI: 10.3390/molecules22040608

Zhu H, Zhang Y, Ye G, Li Z, Zhou P, Huang C. 2009. In Vivo and in Vitro Antiviral Activities of Calycosin-7-O-β-D-glucopyranoside against Coxsackie Virus B3. *Biological & Pharmaceutical Bulletin* 32(1):68-73. DOI: 10.1248/bpb.32.68

Kamrani Y Y, Amanlou M, Esmaeelian B, Bidhendi S M, Sahebjamei M. 2007. Inhibitory Effects of a Flavonoid-Rich Extract of Pistacia vera Hull on Growth and Acid Production of Bacteria Involved in Dental Plaque. *International Journal of Pharmacology* 3(3). DOI: 10.3923/ijp.2007.219.226

Gadkowski W, Siepka M, Janeczko T, Kostrzewa-Susow E, Popoński J, Mazur M, Arowska B, Aba W, Maciejewska G, Wawrzenieczyk C. 2019. Synthesis and Antimicrobial Activity of Methoxy- Substituted γ-Oxa-ε-lactones Derived from Flavanones. *Molecules* 24(22). DOI: 10.3390/molecules24224151

Martin U, Romina G, Priscila C. 2013. Endophytic fungi from Peruvian highland and lowland
habitats form; distinctive and host plant-specific assemblages. *Biodiversity & Conservation* 22(4):999-1016. DOI: 10.1007/s10531-013-0464-x

Carroll G.C, Carroll F.E. 1978. Studies on the incidence of coniferous needle endophytes in the Pacific Northwest. *Canadian Journal of Botany* 56(24):3034-3043. DOI: 10.1139/b78-367

Qiu M, Xie R S, Shi Y, Zhang H, Chen HM. 2010. Isolation and identification of two flavonoid-producing endophytic fungi from *Ginkgo biloba* L.. *Annals of Microbiology*. DOI: 10.1007/s13213-010-0016-5

Shih T C, Tian X, Huang C, Huang J 2017. Identification of flavonoids and flavonoid-producing endophytic fungi isolated from *opisthopappus*. *Bangladesh Journal of Botany* 46(3):1063-1070.

Shou-Jie L, Xuan Z, Xiang-Hua W, Chang-Qi Z. 2019. Novel natural compounds from endophytic fungi with anticancer activity. *European Journal of Medicinal Chemistry* :316-343. DOI: 10.1016/j.ejmech.2018.07.015

Chi W C, Pang K L, Chen W L, Wang G J, Lee T H. 2019. Antimicrobial and iNOS inhibitory activities of the endophytic fungi isolated from the mangrove plant *Acanthus ilicifolius* var. xiamenensis. *Botanical Studies* 60(1). DOI: 10.1186/s40529-019-0252-3

Tang Z, Wang Y, Yang J, Xiao Y, Wang G. 2020. Isolation and identification of flavonoid-producing endophytic fungi from medicinal plant *Conyza blinii* H.Lév that exhibit higher antioxidant and antibacterial activities. *PeerJ* 8:e8978. DOI: 10.7717/peerj.8978

Du H, Wang H, Yu J, Liang C, Li P. 2012. Enrichment and Purification of Total Flavonoid C-Glycosides from Abrus mollis Extracts with Macroporous Resins. *Industrial & Engineering Chemistry Research* 51(21):7349-7354. DOI: 10.1021/ie3004094

Li J, Chen Z, Di D. 2012. Preparative separation and purification of Rebaudioside A from Stevia
rebaudiana Bertoni crude extracts by mixed bed of macroporous adsorption resins. *Food Chemistry* 132(1):268-276. DOI: 10.1016/j.foodchem.2011.10.077

Chen Z, Long J, Kang L, Du X, Di D. 2013. Modified macroporous adsorption resin (LX1180) used to adsorb flavonoid. *Pigment & Resin Technology* 42(6). DOI: 10.1108/PRT-04-2012-0030

Zhang L, Wu T, Wang Z, Ding G, Zhao L. 2018. Enrichment and Purification of Total Ginkgo Flavonoid O-Glycosides from *Ginkgo Biloba* Extract with Macroporous Resin and Evaluation of Anti-Inflammation Activities In Vitro. *Molecules* 23(5):1167. DOI: 10.3390/molecules23051167

Saraswaty V, Srikandace Y, Simbiyani N A, Jasmansyah, Udin Z. 2013. Antioxidant activity and total phenolic content of endophytic fungus *Fennellia nivea* NRRL 5504.. *Pakistan Journal of Biological ences Pjbs* 16(22):1574-8. DOI: 10.3923/pjbs.2013.1574.1578

Tao J, Wei Y, Hu T. 2015. Flavonoids of *Polygonum hydropiper* L. attenuates lipopolysaccharide-induced inflammatory injury via suppressing phosphorylation in MAPKs pathways.. *Bmc Complementary & Alternative Medicine* 16(1):1-15. DOI: 10.1186/s12906-016-1001-8

Bi Y G, Tan Y Q. 2012. Study on Macroporous Resin Separation and Purification of Total Flavonoids of Plantago Process. *Advanced Materials Research* 550-553:987-992. DOI: 10.4028/www.scientific.net/AMR.550-553.987

Zhang L, Wu T, Wei X, Wang Z, Ding G, Zhao L. 2018. Enrichment and Purification of Total Ginkgo Flavonoid O-Glycosides from *Ginkgo Biloba* Extract with Macroporous Resin and Evaluation of Anti-Inflammation Activities In Vitro. *Molecules* 23(5):1167. DOI: 10.3390/molecules23051167
Yu M, Wang B, Qi Z, Xin G, Li W. 2019. Response Surface Method Was Used to Optimize the Ultrasonic Assisted Extraction of Flavonoids from *Crinum asiaticum*. *Saudi Journal of Biological Sciences* 26(8). DOI: 10.1016/j.sjbs.2019.09.018

Kaur N, Arora D S, Kalia N, Laur M. 2020. Antibiofilm, antiproliferative, antioxidant and antimutagenic activities of an endophytic fungus *Aspergillus fumigatus* from Moringa oleifera. *Molecular Biology Reports* 47(4). DOI: 10.1007/s11033-020-05394-7

Tsimogiannis D I, Oreopoulou V. 2006. The contribution of flavonoid C-ring on the DPPH free radical scavenging efficiency. A kinetic approach for the 3’,4’-hydroxy substituted members. *Innovative Food Science & Emerging Technologies* 7(1-2):140-146. DOI: 10.1016/j.ifset.2005.09.001

Zhang H, Yang Y F, Zhou Z Q. 2018. Phenolic and flavonoid contents of mandarin (Citrus reticulata Blanco) fruit tissues and their antioxidant capacity as evaluated by DPPH and ABTS methods. *Journal of Integrative Agriculture* 17(1):256-263. DOI: 10.1016/S2095-3119(17)61664-2

Chobot V, Hadacek F. 2011. Exploration of pro-oxidant and antioxidant activities of the flavonoid myricetin. *Redox Report* 16(6):242-247. DOI: 10.1179/1351000211Y.0000000015

Zhishen J, Mengcheng T, Jianming W. 1999. The determination of flavanoid contents on mulberry and their scavenging effects on superoxide radical. *Food Chemistry* 64(4):555-559. DOI: 10.1016/S0308-8146(98)00102-2

Leong C N A, Tako M, Hanashiro I, Tamaki H. 2008. Antioxidant flavonoid glycosides from the leaves of *Ficus pumila* L. *Food Chemistry* 109(2):415-420. DOI: 10.1016/j.foodchem.2007.12.069
Wu DQ, Ma ZY, Hei JW, Li S. 2017. Antioxidant Stability of flavonoid from Oriental Stephania Root. *West China J Pharm SCI* 32(5):511-513. DOI: 10.13375/j.cnki.wcjps.2017.05.019

Li YM, Gao J, Yang ZJ, Lin ML. 2007. Studt on the Adsorbing and Refining Alkaloid from *Cynanchum komaroviiai. Iljinoki* with Macro-porous Resin. *J Inner Mongolia Univ of Tech: Nat Sci Ed* 26(1):38-41. DOI: 10.3969/j.issn.1001-5167-B.2007.01.008

Khled Khoudja N, Boulekbache-Makhlouf L, Madani K. 2014. Antioxidant capacity of crude extracts and their solvent fractions of selected Algerian Lamiaceae. *Industrial Crops and Products* 52:177-182. DOI: 10.1016/j.indcrop.2013.10.004

Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH. 2012. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition & Analysis* 19(6-7):669-675. DOI: 10.1016/j.jfca.2006.01.003

Sakanaka S, Tachibana Y, Okada Y. 2005. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). *Food Chemistry* 89(4):569-575. DOI: 10.1016/j.foodchem.2004.03.013

Ozsoy N, Can A, Yanardag R & Akev N. 2008. Antioxidant activity of Smilax excelsa L. leaf extracts. *Food Chemistry* 110(3):571-583. DOI: 10.1016/j.foodchem.2008.02.037

Aruoma OI, Grootveld M, Bahorun T. 2006. Free radicals in biology and medicine: from inflammation to biotechnology. *Biofactors* 27(1-4):1-3. DOI:10.1002/biof.5520270101

Baran M Y, Güzin Emecen, András Simon, Góbor Tóth, Kuruuzum-Uz A. 2020. Assessment of the antioxidant activity and genotoxicity of the extracts and isolated glycosides with a new flavonoid from Lotus aegaeus (Gris.) Boiss. *Industrial Crops and Products* 153:112590. DOI: 10.1016/j.indcrop.2020.112590
Zou Y, Xin X, Xu H, Yuan H, Zhao G. 2020. Highly efficient bioconversion of flavonoid glycosides from citrus-processing wastes in solvent-buffer systems. *Green Chemistry* 22(Pt 3). DOI: 10.1039/D0GC00669F

Zhang XH, Shen J, Zhao CC, Shao JH. 2020. A New Flavonoid Glycoside with α-Glucosidase Inhibitory Activity from *Galium Verum*. *Chemistry of Natural Compounds* 56(1):1-3. DOI: 10.1007/s10600-020-02945-z

Taghinia P, Khodaparast M H H, Ahmadi M. 2019. Free and bound phenolic and flavonoid compounds of Ferula persica obtained by different extraction methods and their antioxidant effects on stabilization of soybean oil. *Journal of Food Measurement and Characterization* 2019(2). DOI: 10.1007/s11694-019-00218-0

Lee KW, Lee HJ. 2006. Biphasic effects of dietary antioxidants on oxidative stress-mediated carcinogenesis. *Mechanisms of Ageing & Development* 127(5):424-431. DOI: 10.1016/j.mad.2006.01.021

Zhang CH, Yu Y, Liang YZ, Chen XQ. 2015. Purification, partial characterization and antioxidant activity of polysaccharides from *Glycyrrhiza uralensis*. *International Journal of Biological Macromolecules* 79:681-686. DOI: 10.1016/j.ijbiomac.2015.05.060
Figure 1

Relationship between adsorption time and adsorption rate

The data point indicates the change of adsorption rate over time.
Figure 2

Adsorption of D101 under different pH conditions

The data point indicates the change of adsorption rate over pH.
Figure 3

Adsorption of D101 under different liquid temperature

The data point indicates the change of adsorption rate over temperature.
Figure 4

Response Surface of interrelated influence of temperature and pH to flavonoids rate
Figure 5

Response Surface of interrelated influence of temperature and time to flavonoids rate
Figure 6

Response Surface of interrelated influence of pH and time to flavonoids rate
Figure 7

Comparison of elution rate and purity of total flavonoids at different concentrations

The data point indicates the change of elution rate and purity of flavonoid over eluent.
Figure 8

Scavenging ability of total flavonoids of endophytic fungi on DPPH

Each data point indicates the clearance rate of total flavonoids on DPPH before and after extraction where Vc is the control.
Figure 9

Scavenging ability of total flavonoids of endophytic fungi on ABTS

Each data point indicates the clearance rate of total flavonoids on ABTS before and after extraction where Vc is the control.
Figure 10

Scavenging ability of total flavonoids of endophytic fungi on -OH

Each data point indicates the clearance rate of total flavonoids on -OH before and after extraction, where Vc is the control.
Figure 11

Scavenging ability of total flavonoids of endophytic fungi on •O₂⁻

Each data point indicates the clearance rate of total flavonoids on •O₂⁻ before and after extraction where Vc is the control.
### Table 1 (on next page)

Factors and the levels of experiment of Response Surface Analysis
| Factors       | Factor levels |
|--------------|---------------|
| Temperature/°C | 25 30 35      |
| pH           | 2.5 3.5 4.5   |
| time/h       | 0 1 2         |
Table 2 (on next page)

Observed and estimated values for different levels of experimental design
Table 2 Observed and estimated values for different levels of experimental design

| No. | A   | B   | C   | Adsorption rate/% |
|-----|-----|-----|-----|-------------------|
| 1   | 0   | 0   | 0   | 0.4739            |
| 2   | 0   | 1   | 1   | 0.485             |
| 3   | 0   | 0   | 0   | 0.4709            |
| 4   | 1   | 0   | -1  | 0.448             |
| 5   | 0   | -1  | -1  | 0.4667            |
| 6   | 1   | 1   | 0   | 0.3244            |
| 7   | 1   | -1  | 0   | 0.2403            |
| 8   | -1  | -1  | 0   | 0.5625            |
| 9   | -1  | 0   | -1  | 0.4396            |
| 10  | -1  | 1   | 0   | 0.2957            |
| 11  | 0   | 0   | 0   | 0.4739            |
| 12  | 0   | 0   | 0   | 0.4402            |
| 13  | 0   | 1   | -1  | 0.3885            |
| 14  | 0   | -1  | 1   | 0.5616            |
| 15  | -1  | 0   | 1   | 0.555             |
| 16  | 0   | 0   | 0   | 0.4739            |
| 17  | 1   | 0   | 1   | 0.3927            |
### Table 3 (on next page)

Analyze of mean square

SS means sum of squares; DF means degree of freedom; MS means mean square; F means a statistic obtained by analysis of variance based on experimental data; Prob>F means the chance that an F this large could occur due to noise.
Table 3: Analyze of mean square

| Source      | SS     | DF | MS    | F       | > F    | Prob |
|-------------|--------|----|-------|---------|--------|------|
| Model       | 0.1236 | 9  | 0.0137| 17.2796 | 0.0005 |
| A-temperature| 0.0250 | 1  | 0.0250| 31.4807 | 0.0008 |
| B-pH        | 0.0142 | 1  | 0.0142| 17.9143 | 0.0039 |
| C-time      | 0.0079 | 1  | 0.0079| 9.9478  | 0.0161 |
| AB          | 0.0308 | 1  | 0.0308| 38.7302 | 0.0004 |
| AC          | 0.0073 | 1  | 0.0073| 9.1654  | 0.0192 |
| BC          | 0.0000 | 1  | 0.0000| 0.0008  | 0.9782 |
| A^2         | 0.0171 | 1  | 0.0171| 21.5163 | 0.0024 |
| B^2         | 0.0093 | 1  | 0.0093| 11.7547 | 0.0110 |
| C^2         | 0.0132 | 1  | 0.0132| 16.6103 | 0.0047 |
| Error       | 0.0056 | 7  | 0.0008|         |        |
| Lack of Fit | 0.0047 | 3  | 0.0016| 7.1415  | 0.0439 |
| Pure Error  | 0.0009 | 4  | 0.0002|         |        |
| Total       | 0.1292 | 16 |       |         |        |