An Optimized Method for Extracting Oenothein B from *Eucalyptus* Leaves

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Eucalyptus is a fast-growing and high-yield tree species producing approximately one third of the timber of the world. *Eucalyptus* leaves are a by-product of timber with comparable biomass, and are largely unused. *Eucalyptus* leaves are rich in polyphenols, of which oenothein B is the most abundant. In this study, the authors developed an ultrasonic-based method for extracting oenothein B from *Eucalyptus* leaves. The ethanol concentration was proven to be a key determinant for the extraction efficiency and quality of oenothein B. Extracting with an ethanol concentration greater than 20% resulted in the altered chemical structure of oenothein B. The optimized conditions for Oenothein B extraction from *Eucalyptus* leaves used 10% ethanol and a 1 to 50 (g/mL) material to liquid ratio for 1 h under 40 kHz ultrasonic at a temperature of 87 °C. The highest extraction yield obtained was 12.4%. The oenothein B extract showed the capability of reactive oxygen scavenging. The accumulation pattern of oenothein B during the developmental processes of *Eucalyptus* leaves was detected using the developed method, and the rapid-accumulation period of oenothein B was determined, which will facilitate the utilization of *Eucalyptus* leaf resources.

DOI: 10.15376/biores.17.4.6094-6107

Keywords: Oenothein B (OeB); Eucalyptus leaves; Ultrasonic extraction; Ethanol; Antioxidant

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INTRODUCTION

Eucalypt is the general name of the species of genus *Angophora*, *Corymbia*, and *Eucalyptus* of the Myrtaceae family, containing a total of 945 species and varieties (Xie 2019). *Eucalyptus*, as one of the representative fast-growing tree species in the world, has the following characteristics: fast growth rate and short rotation period. It is widely used in the following industries: papermaking, furniture, and wood-based artificial plate, *etc*. (Qi 2006; Ding *et al*. 2021). *Eucalyptus* is a deciduous tree species. In addition to timber production, considerable biomass is also produced in *Eucalyptus* leaves every year. Looking at the Guangxi province of China as an example, the total output of *Eucalyptus* timber was greater than 30 million m^3, and the total production of *Eucalyptus* leaves was approximately 2500 tons in 2020. However, these biological resources are not only unused, but they also consume additional costs in waste disposal. *Eucalyptus* leaves contain a large number of secondary metabolites with bioactivities, which are important resources to be developed and utilized (Mu 2021). Therefore, the development and utilization of *Eucalyptus* leaves has great potential and is also an important supplement to the *Eucalyptus* industry.

Eucalyptus leaves are rich in acyl phenols and derivatives, flavonoids, and tannins (Cao *et al*. 2016a). Among them, oenothein B (OeB) has been identified as one of the most abundant secondary metabolites accumulated in *Eucalyptus* leaves (Bazylko *et al*. 2007; Schepetkin *et al*. 2009; Yoshida *et al*. 2018). Oenothein B is an ellagic acid tannin composed of two tellimagrandin I units, which form a macrocyclic dimer through binding with glucose and gallic acyl groups *via* ester bonds and glycoside bonds (Fig. 1) (Santos *et al*. 2014; Li *et al*. 2020).

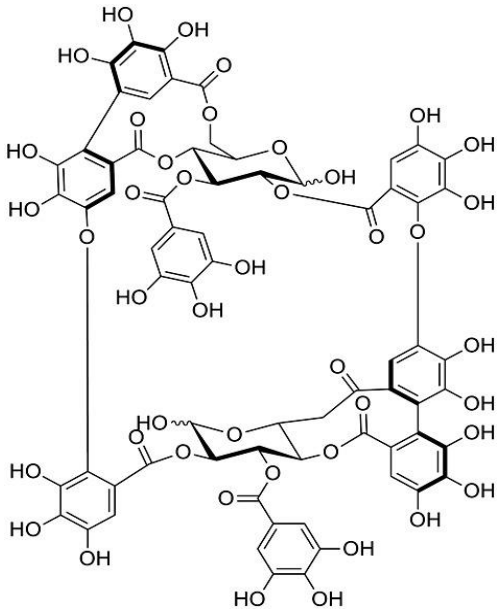

Fig. 1. The chemical structure of OeB

Oenothein B has been reported as having high antioxidant activity, and it considerably reduces the content of reactive oxygen species (ROS) by inhibiting the activities of hyaluronidase and peroxidase *in vivo* (Toth *et al*. 2009). In addition to antioxidant activity, OeB also shows an inhibitory effect on a variety of tumor cells. For example, OeB can inactivate the ROS induced PI3K/Akt/NF-κB pathway to restrict the cells in the G_1 stage, thus it effectively inhibits the proliferation of lung cancer cell A549 (Pei *et al*. 2018). The growth of breast cancer cell lines MCF7, MDA-MB-468, and MDA-MB-231 was inhibited by OeB in low concentration and ceased in high concentration (Maruška *et al*. 2017). Oenothein B also shows antiviral and anti-inflammatory activity; thus, it has been used for the therapy of benign prostatic hyperplasia, urethritis, gastric ulcers, and other diseases (Kadam *et al*. 2018; Dacrema *et al*. 2020; Esposito *et al*. 2021). For example, OeB extracted from *Oenothera glazioviana* effectively inhibited the invasion of the hepatitis C virus (HCV) (Satoru *et al*. 2019). In addition, OeB plays a role in neuroprotection and body immunity improvement, which might function through activating the extracellular signal regulated kinase 2 in the brain or enhancing the production of human interferon γ (IFNγ) in T cells (Amakura *et al*. 2009; Kiss *et al*. 2011; Ramstead *et al*. 2012; Okuyama *et al*. 2021). Taken together, OeB is a plant secondary metabolite with a variety of biological activities, and the high accumulation of OeB in *Eucalyptus* leaves shows great potential. At present, plant extracts with high content of OeB are mainly used for clinical research and treatment of diseases, for example, the

extract of *Epilobium angustifolium* L. (Esposito *et al*. 2021; Maruška *et al*. 2017). Although OeB extract is effective in treating diseases, almost none of the purified OeB was used. It can be seen that it is necessary to separate OeB.

To better use *Eucalyptus* resources, in this study, the authors developed an ultrasonic-based, simple and low-cost method to extract OeB from *Eucalyptus* leaves. The effects of various parameters, including the solvent concentration, temperature, material to liquid ratio, and extraction time, on the extraction efficiency and product quality were analysed and optimized.

EXPERIMENTAL

Materials

The experimental materials were collected from the clonal forest of *Eucalyptus urophylla* × *E. grandis* grown in the Southern Seedling Base of the China Eucalyptus Research Centre, Zhanjiang City, Guangdong Province. The fresh and healthy leaves of 3-year-old *Eucalyptus urophylla* × *E. grandis* with CEPT 1774 were selected, dried at a temperature of 60 °C for 4 h, and then stored in a -70 °C refrigerator for future use. The leaves of CEPT 1774 grow at an annual average temperature of 23.2 °C, annual sunshine hours of 1714 to 2038 h, an annual total solar radiation of 102 to 118 Kcal/cm^2, and an annual average rainfall of 1417 to 1802 mm.

Chromatographic Conditions

The chromatographic column was a Diamonsil C18 (2) column (5 μm 250 mm × 4.6 mm). The organic phase was methanol, the aqueous phase was 0.1% formic acid, the column temperature was 30 °C, the injection volume was 10 μL, the flow rate was 1 mL/min, the detection wavelength was 270 nm, the gradient elution program was 0 min to 35 min, the methanol was 10% to 50%, 0.1% formic acid was 90% to 50%; 30 min to 35 min, methanol was 10%, and 0.1% formic acid was 90% (Cao *et al*. 2016b). The sample was detected *via* a U3000 ultra-high performance liquid chromatograph (Thermo Fisher, Waltham, MA, USA).

Mass Spectroscopy (MS) Conditions

The column was a Waters Acquity UPLC BEH C18 column (1.7 μm x 2.1 mm × 50 mm). The organic phase was acetonitrile, the aqueous phase was 0.1% formic acid, the injection volume was 10 μL, the flow rate was 0.4 mL/min, the ion source was ESI, the scanning mode was multiple reaction detection (MRM), and the samples were detected *via* a 6500 Q-TOF instrument (Agilent Technologies, Santa Clara, CA).

Determination of the Oenothein B (OeB) Extraction Yield

Sample preparation

Approximately 10 mg of *Eucalyptus* leaf powder was used for liquid phase detection, with different ethanol concentrations, extraction times (min), solid to liquid ratios (g/mL), extraction temperatures (°C), and ultrasonic cleaning at 40 kHz. It was then extracted for 1 h, centrifuged at 4000 r/min for 10 min, the supernatant was removed and passed through a 0.22-μm filter, and then stored at a temperature of 4 °C for subsequent detection.

Preparation of the standard curve

The OeB standard product was provided by the School of Food Science of South China Agricultural University. The standard curve was drawn with the concentration of OeB standard solution (mg/mL) as the X-axis and the peak area (mAu*min) as the Y-axis (linear range 0.0125 to 1 mg/mL).

Calculation

The OeB extraction yield was calculated according to Eq. 1,

$$\text{OeB extraction rate } (\%) = \frac{c \times V}{m} \times 100\% \quad (1)$$

where c is the concentration of OeB (mg/mL), *v* is the volume of the extract (mL), and *m* is the mass of *Eucalyptus* leaves powder (mg).

Orthogonal experimental design and analysis of variance

On the basis of a single factor experiment, the ethanol concentration (A), extraction temperature (B), and solid-liquid ratio (C) were selected as the factors to be investigated, and a L9 (3^4) orthogonal experiment was designed to study the extraction process of *Eucalyptus* OeB (as shown in Table 1).

Table 1. Factor and Level of the Orthogonal Test

Level	Factors		
	A (%)	B (°C)	C (g/mL)
1	5	80	1:30
2	10	87	1:40
3	15	94	1:50
Note: A is the ethanol concentration, B is the extraction temperature, and C is the solid-liquid ratio			

Assessment of Antioxidant Activity

First, the sample stock solution was diluted 8-fold to a concentration of 312.5 μg/mL, and then sequentially halved to 5 sample working solutions of different concentrations, and the scavenging ability of these samples against 1,1-diphenyl-2-picrylhydrazyl (DPPH)/2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonate) (ABTS) radicals was determined according to Eq. 2,

$$\text{DPPH/ABTS radical scavenging ratio } (\%) = \left(1 - \frac{A_S}{A_C}\right) \times 100\% \quad (2)$$

where A_S is the absorbance value of the sample tube to be tested, and A_C is the absorbance value of the blank control tube (Li *et al.* 2021; Zhang 2021).

Statistical analysis

One-way ANOVA was performed on the mean of the experimental data using SPSS ver. 25.0 for Windows (SPSS Inc., Chicago, IL, USA) using the honestly significant difference test of LSD, Duncan's test, and Dunnett's test. The level of significance was set to a p-value less than 0.05.

RESULTS AND DISCUSSION

Effect of Ethanol Concentration on Extraction Efficiency of Oenothein B (OeB)

According to its chemical structure, OeB is easily soluble in polar solvents. The authors compared the extraction results among different polar solvents including water, methanol, ethanol, and isopropyl alcohol. The results showed that OeB has the highest solubility in water, while ethanol can improve the purity of the extracting product (data not shown). In addition, the change of the OeB extraction rate was compared under the conditions of no ultrasound (6.2% $\pm$0.04) and ultrasound (8.7% $\pm$ 0.03), and the results showed that ultrasound significantly ameliorated the extraction yield of OeB (Fig. S1). The ethanol concentration was reported to greatly affect the extraction amount. For example, the extraction amount of ginsenosides reached the highest (74.3 mg/g) with 55% ethanol. However, the extraction amount was greatly decreased when lower or higher ethanol concentration were used (Yuan *et al*. 2021). Therefore, different ethanol concentrations were adopted for the OeB extraction. The extracts obtained with ethanol concentration less than 20% showed a single absorption peak, while the products showed a double absorption peak when extracted at 20% or higher ethanol concentrations. The extracts showed a mixed absorption peak when the ethanol concentration reached 50%, which indicated that a high concentration of ethanol introduced impurities or changed the chemical structure of OeB (as shown in Fig. 2).

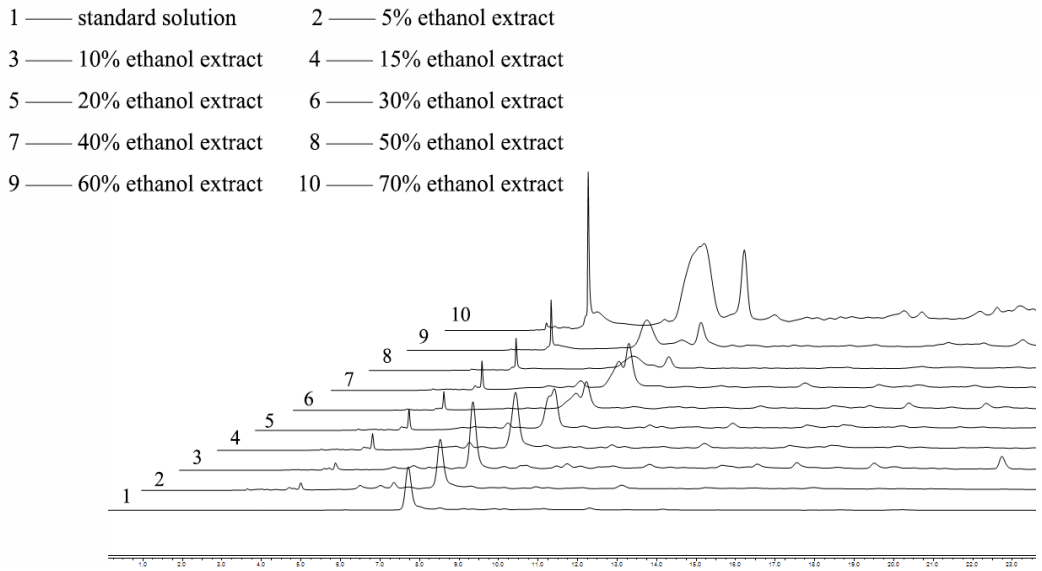

Fig. 2. Extraction effect of OeB with different ethanol concentrations (standard solution dissolved in 10% ethanol)

After treating OeB with low-concentration sulfuric acid (0.05 mol/L), the macrocyclic structure of OeB was destroyed, and two isomers with *m*/*z* of 1591 formed (Hatano *et al*. 1990). To further reveal the effects of the ethanol concentration on OeB, the authors performed time of flight mass spectrometry (TOF MS) analysis on the extracts obtained at different ethanol concentrations (10%, 30%, and 60%).

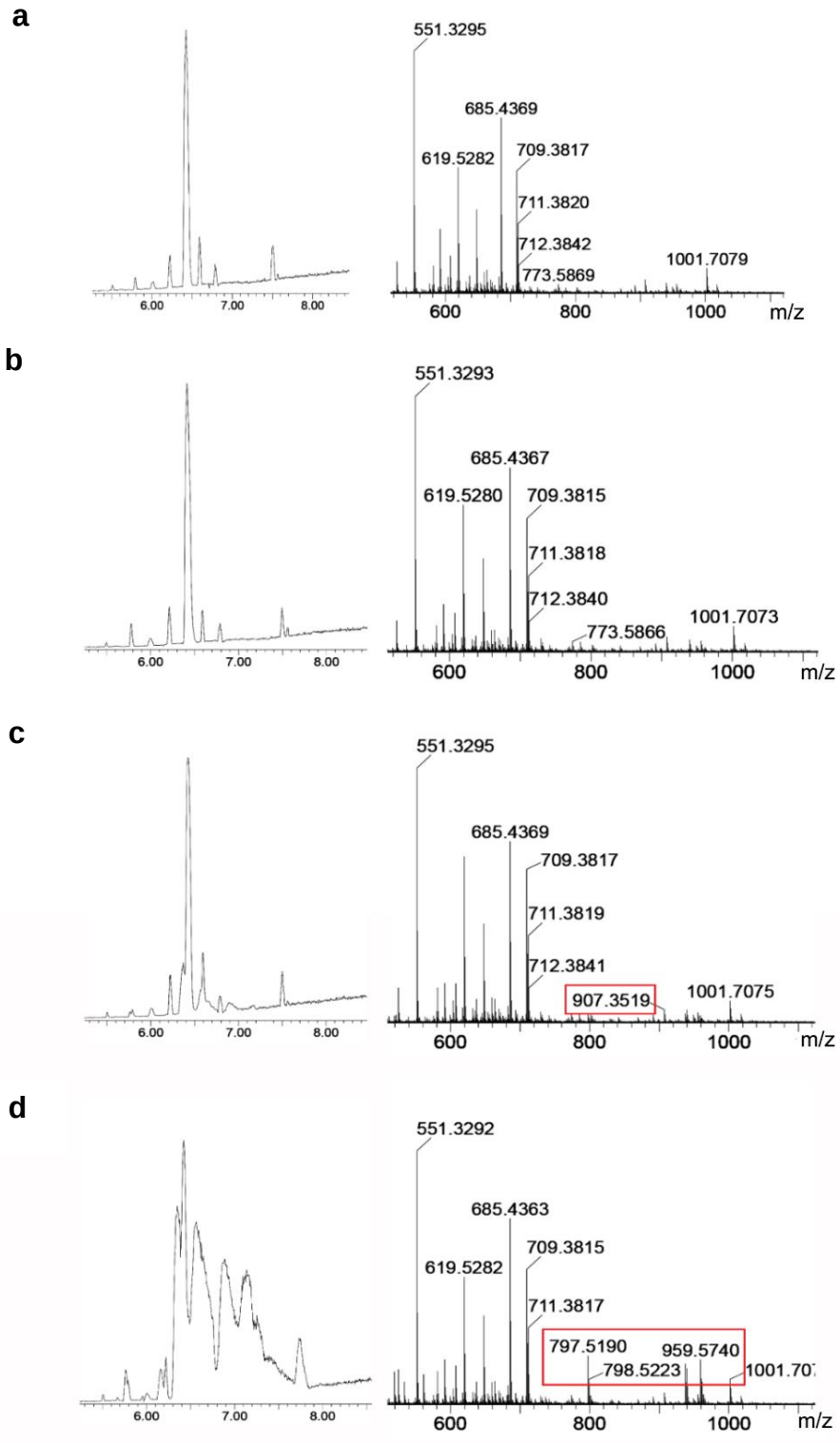

Fig. 3. Total ions chromatogram (left) and mass spectrometry (right) detection of OeB extracts with different ethanol concentrations. The OeB standard product dissolved in: 10% ethanol (a); OeB extracted by 10% ethanol (b); OeB extracted by 30% ethanol (c); and OeB extracted by 60% ethanol (d) (Note: The abscissa is time in total ions chromatogram, and the numbers shown in the mass spectrometry are molecular weights)

The structure of OeB is an asymmetric and difficult-to-separate macrocyclic structure, which is difficult to analyse (Bazylko *et al*. 2007). Therefore, the comparison of the components was based on the molecular weight difference among these extracts. The results demonstrated that a differential component with a molecular weight of 907 was detected in the 30% ethanol extracts, while three differential components with molecular weights of 797, 798, and 959, were detected in the 60% ethanol extracts, and there was no difference was detected between the 10% ethanol extracts and standard sample (as shown in Fig. 3). These results suggested that a high concentration of ethanol might alter the chemical structure of OeB, thus reducing the purity of the OeB extracts. The threshold for the ethanol concentration to ensure the extraction quality of OeB is 20%. Afterward, the authors assessed the extraction yield of OeB at 5%, 10%, and 15% ethanol. The results showed that the extraction yield of OeB reached the highest value (11.1%) when 10% concentration of ethanol was adopted (Fig. 4a). Therefore, 10% was determined as the optimal ethanol concentration for further study.

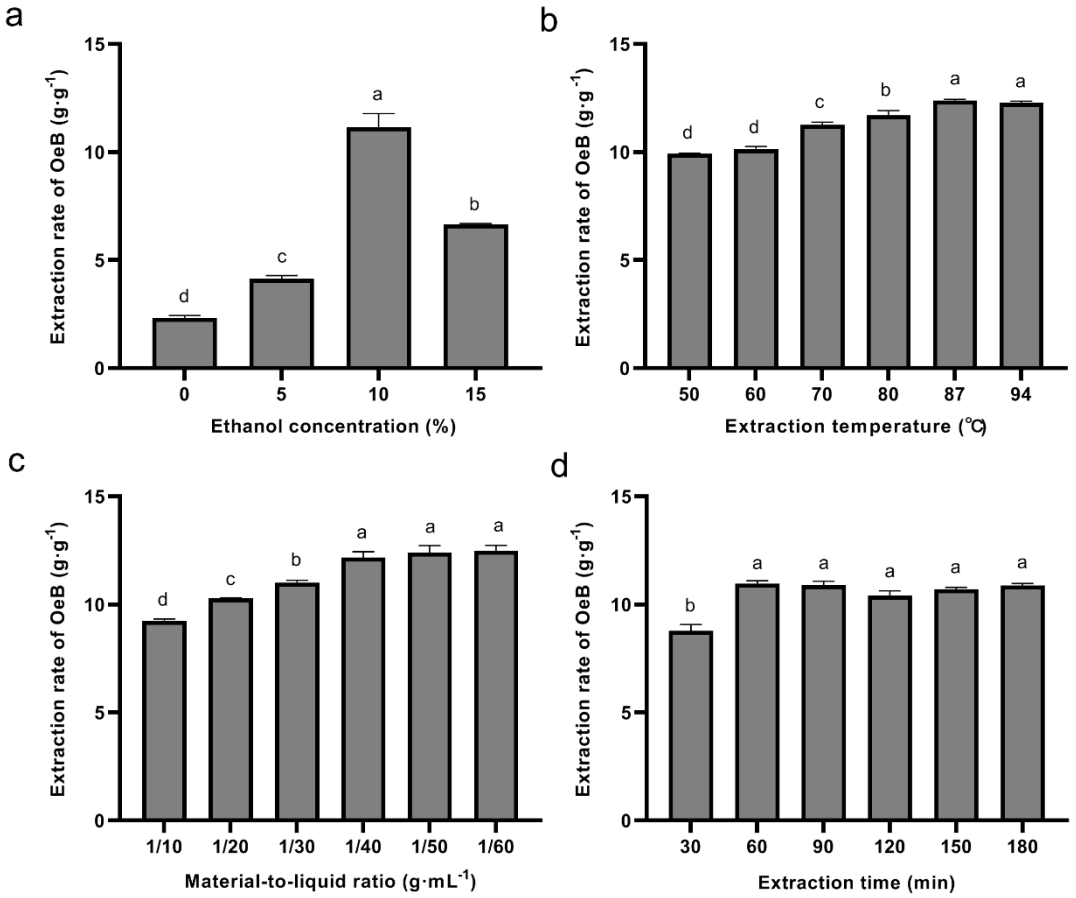

Fig. 4. Effect of different factors on the OeB extraction yield: the effect of the ethanol concentration on the extraction of OeB (a); the effect of time on the extraction of OeB (b); the effect of the temperature on the extraction of OeB (c); and the effect of the material to liquid ratio on the extraction of OeB (d)

Effect of the Temperature, Material to Liquid Ratio, and Extraction Time on the Extraction Efficiency of Oenothein B (OeB)

The ultrasonic extraction method is widely used because of its simplicity, high efficiency, and low amount of harm. Some studies have used ultrasonic-assisted extraction to extract polyphenols from Lvmaofeng tea. Compared to the extraction yield obtained by conventional water extraction (10.9%), the extraction yield of ultrasonic alcohol extraction was considerably improved (14.5%) (Wang *et al*. 2021).

Temperature is an important parameter for OeB extraction. Therefore, the authors set the extraction temperature range from 50 to 94 °C. The extraction yield of OeB increased as the temperature increased from 50 to 87 °C, while no major increase was seen when the extraction temperature was greater than 87 °C (Fig. 4b). For the material to liquid ratio, when it ranged from 1 to 10 to 1 to 40, the extraction yield of OeB gradually increased, while no major improvement was observed when the material to liquid ratio was greater than 1 to 40 (Fig. 4c). In addition, the extraction yield of OeB increased as the extraction time was extended, but it did not further increase after 1 h (Fig. 4d).

Optimized Method for OeB Extraction Determined Through Orthogonal Analysis

According to the existing results, the ethanol concentration, temperature, and material to liquid ratio are critical factors for determining the extraction efficiency of OeB. Therefore, the authors performed an orthogonal test on these parameters (Table 1). The factor priority order for OeB extraction is the ethanol concentration, followed by the material to liquid ratio and temperature. Under the conditions of the predicted optimum combination ($A_2B_2C_3$), the actual extraction yield of OeB from *Eucalyptus* leaves equaled 12.4% (Table 2). The ANOVA analysis confirmed that the ethanol concentration was the most critical factor for extraction efficiency of OeB (Table 3). Using deionized water, the aerial part of willow leaf was extracted with a solid to liquid ratio of 1 to 100 (g/mL), and an extraction yield of OeB was approximately 7.3% as reported in Granica *et al*. 2012. Compared with this, the OeB extraction effect of this study was further improved.

Table 2. Orthogonal Test for Extraction

Level	Factors			Extraction Yield % ($g \cdot g^{-1}$)
	A (%)	B (°C)	C (g/mL)	
1	1	1	1	5.6
2	1	2	2	5.8
3	1	3	3	5.6
4	2	1	2	11.4
5	2	2	3	12.4
6	2	3	1	11.4
7	3	1	3	10.1
8	3	2	1	9.3
9	3	3	2	10.4
K_1	5.7	9.1	8.8	
K_2	11.8	9.2	9.2	
K_3	10.0	9.2	9.5	
R	6.1	0.1	0.7	
Factor priority A > C > B				
Best combination $A_2 B_2 C_3$				
Note: A is ethanol concentration, B is extraction temperature, C is solid-liquid ratio				

Table 3. The ANOVA Analysis Results of Variance

Source of Variance	Deviation Sum of Squares	Degree of Freedom (d_f)	*F* value	Significance
A	58.414	2	29.207	0.012 (< 0.05)
B	0.025	2	0.013	0.964 (> 0.05)
C	0.540	2	0.270	0.558 (> 0.05)
Error value	0.683	2		
Note: A is ethanol concentration, B is extraction temperature, C is solid-liquid ratio				

The Antioxidant Activity of the OeB Extracts

Plants, vegetables, and fruits are rich in polyphenols, which block their own excessive free radical responses to combat adverse environments (Sharma *et al*. 2019). Phenolic compounds can form coupling compounds with 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonate) (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals, and their antioxidant activity can be determined based on the degree of decolorization (Gulcin İ 2020; Ilyasov *et al*. 2020).

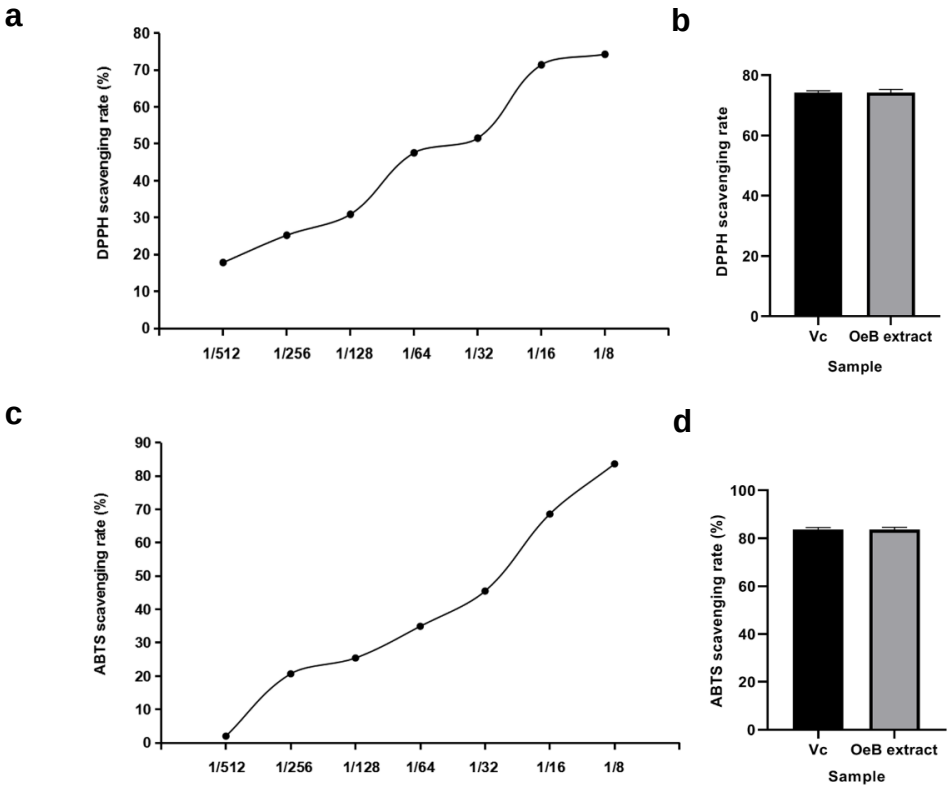

Fig. 5. Scavenging activity of OeB extract against DPPH and ABTS free radical: the scavenging extent of OeB extract on DPPH free radical (**a**); comparison of ABTS free radical scavenging extent between Vc and the 312.5 μg/mL concentration sample (**b**); the scavenging extent of OeB extract on DPPH free radical (**c**); and the comparison of ABTS free radical scavenging extent between Vc and the 312.5 μg/mL concentration sample (**d**) (Note: 1/8 = 312.5 μg/mL; 1/16 = 156.3 μg/mL; 1/32 = 78.1 μg/mL; 1/64 = 39.1 μg/mL; 1/128 = 19.5 μg/mL; 1/256 = 9.8 μg/mL; 1/512 = 4.9 μg/mL; and Vc = 8.0 μg/mL)

Antioxidant activity is a basic attribute of OeB; thus the authors measured the antioxidant activity of the OeB extracts obtained through this optimized method. The scavenging percentage on the free radicals of OeB extracts were 74.3% ± 0.1% and 83.6% ± 0.1%, indicated by DPPH and ABTS, respectively (as shown in Fig. 5a and 5c). The activity of the scavenging free radicals of OeB is comparable to that of Vc (as shown in Fig. 5b and 5d). Onion polyphenols were extracted with 75% ethanol and when the concentration of onion polyphenols was 0.3 mg/mL, the scavenging rate of the DPPH free radicals reached 86.2% (Cao *et al.* 2020). Therefore, it was suggested that the extract obtained by this method retained antioxidant activity of OeB.

The Accumulation Pattern of OeB in Eucalyptus Leaves During Development

Eucalyptus leaves at 8 different developmental stages were collected to detect the OeB content using the optimized conditions (as shown in Fig. 6a). The OeB content increased along with leaf development. The OeB content dramatically increased at stages T1 through T3, stabilized in mature *Eucalyptus* leaves during the T4 through T7 stages, and decreased in aged *Eucalyptus* leaves at the stage T8 (as shown in Fig. 6b). The pattern of OeB accumulation in *Eucalyptus* leaves indicated that molecular regulation of OeB biosynthesis is initiated at T1.

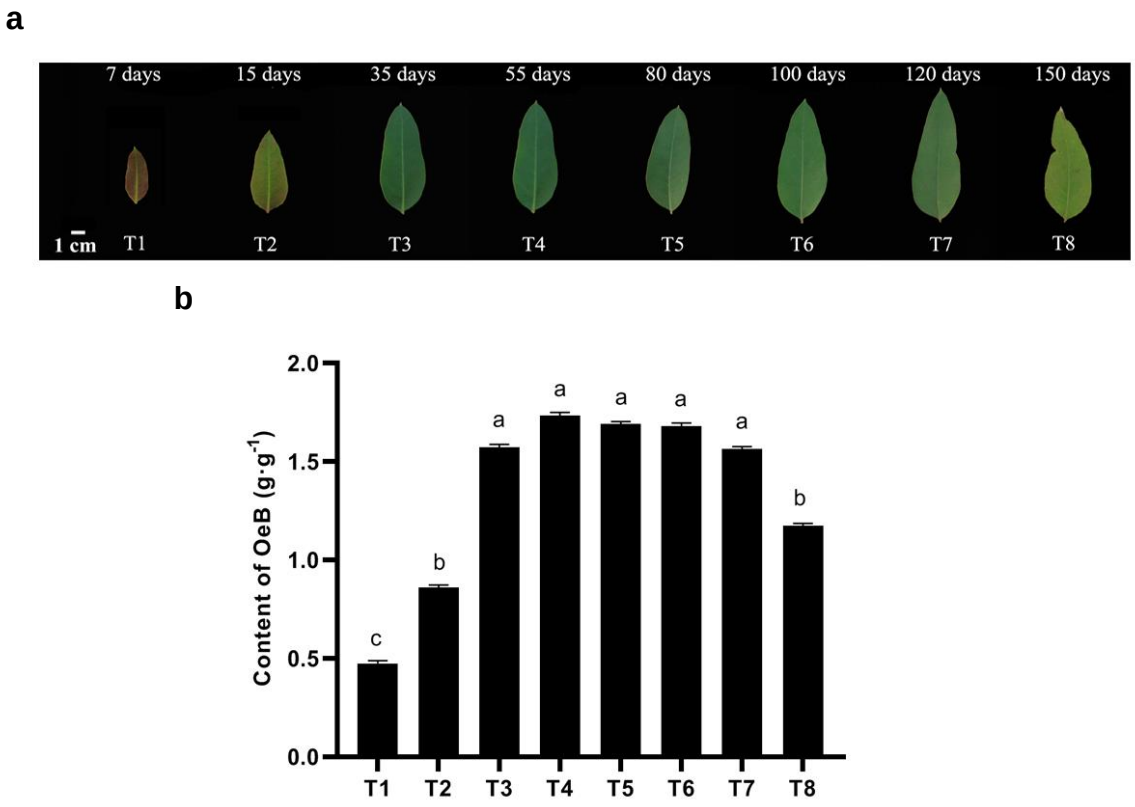

Fig. 6. *Eucalyptus* leaves with different leaf ages and OeB content

CONCLUSIONS

1. Ethanol concentration is a key factor affecting oenothein B (OeB) extraction yield of *Eucalyptus* leaves. Low concentration (< 20%) is conductive to the dissolution of *Eucalyptus* OeB.
2. The optimized conditions for OeB extraction from *Eucalyptus* leaves were determined to be as follows: 10% ethanol, a 1 to 50 (g/mL) material to liquid ratio, 40 kHz ultrasonic treatment, and a temperature of 87 °C for 1 h.
3. The 8 times diluted *Eucalyptus* OeB reached 74.3% and 83.6% of DPPH and ABTS free radicals, respectively, showing strong antioxidant ability.
4. The OeB content dramatically increased at early growth period (TI~T3), stabilized in mature *Eucalyptus* leaves (T4~T7), and decreased in aged *Eucalyptus* leaves (T8).

ACKNOWLEDGMENTS

This work was financially supported by the Key Research Projects of Hunan Education Department (Grant No. 20A521), the Natural Science Foundation of Hunan Province (Grant No. 2021JJ31144), and the Innovation Project of Forestry Science and Technology in Hunan Province (Grant No. XLK201984).

Author Contributions

Meng Li: guided the experimental; Yating Deng: wrote the article, methodology, and investigation; Ziyi Tan, Xiaoqi Dang, Rongjie Mao, and Siyu Long: processed the *Eucalyptus* raw material; and Wei Li: provided the OeB standard products. All authors have read and agreed to the published version of the manuscript.

Conflict Of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

REFERENCES CITED

Amakura, Y., Yoshimura, M., Sugimoto, N., Yamazaki, T., and Yoshida, T. (2009). "Marker constituents of the natural antioxidant *Eucalyptus* leaf extract for the evaluation of food additives," *Bioscience, Biotechnology, and Biochemistry* 73(5), 1060-1065. DOI: 10.1271/bbb.80832

Bazylko, A., Kiss, A. K., and Kowalski, J. (2007). "High-performance thin-layer chromatography method for quantitative determination of oenothein B and quercetin glucuronide in aqueous extract of *Epilobii angustifolii herba*," *Journal of Chromatography A* 1173(1-2), 146-150. DOI: 10.1016/j.chroma.2007.10.019

Cao, S., Chen, Y., Chen, H., Ouyang,W., Ye, X., Cao, Y., and Zhao L. (2016a). "Preparation and *in vitro* and *in vivo* stability of oenothein B from *Eucalyptus* leaves," *Chemistry and Industry of Forest Products* 36(1), 112-118. DOI: 10.3969/j.issn.0253-2417.2016.01.016

Cao, Y., Chen, Y. J., Li, J., Tan, R. W., Cao, S. F., Yu, Z., and Li, W. (2016b). "Research progress on secondary metabolites of *Eucalyptus*," *Eucalypt Science Technology* 33(1), 43-48. DOI: 10.3969/j.issn.1674-3172.2016.01.008

Cao, Y. H., Chen, L. L., and Zhang, X. F. (2020). "Study on extraction of polyphenol from onion by response surface methods and antioxidant activity," *Food Research and Development* 41(12), 164-170. DOI: 10.12161/j.issn.1005-6521.2020.12.027

Dacrema, M., Sommella, E., Santarcangelo, C., Bruno, B., Marano, M. G., Insolia, V., Saviano, A., Campiglia, P., Stornaiuolo, M., and Daglia, M. (2020). "Metabolic profiling, in vitro bioaccessibility and in vivo bioavailability of a commercial bioactive Epilobium angustifolium L. extract," *Biomedicine & Pharmacotherapy* 131(4), article no. 110670. DOI: 10.1016/j.biopha.2020.110670

Ding, J. B., Chen, S. P., and Mo, T. J. (2021). "Research on the management countermeasures of *Eucalyptus* young forest and mature forest tending," *South China Agriculture* 15(8), 70-71. DOI: 10.19415/j.cnki.1673-890x.2021.08.034

Esposito, C., Santarcangelo, C., Masselli, R., Buonomo, G., and Daglia, M. (2021). "*Epilobium angustifolium* L. extract with high content in Oenothein B on benign prostatic hyperplasia: A monocentric, randomized, double-blind, placebo-controlled clinical trial," *Biomedicine & Pharmacotherapy* 138(23), article no. 111414. DOI: 10.1016/j.biopha.2021.111414

Granica, S., Bazylk, A., and Kiss, A. K. (2012). "Determination of macrocyclic ellagitannin Oenothein B in plant materials by HPLC-DAD-MS: Method development and validation," *Phytochemical Analysis* 23(6), 582-587. DOI: 10.1002/pca.2358

Gulcin, İ. (2020). "Antioxidants and antioxidant methods: an updated overview," *Archives of Toxicology* 94(3), 651-715. DOI: 10.1007/s00204-020-02689-3

Hatano, T., Yasuhara, T., Matsuda, M., Yazaki, K., Yoshida, T., and Okuda, T. (1990). "Oenothein B, a dimeric, hydrolysable tannin with macrocyclic structure, and accompanying tannins from *Oenothera erythrosepala*," *Journal of the Chemical Society, Perkin Transactions 1* (10), 2735-2743. DOI: 10.1039/P19900002735

Ilyasov, I. R., Beloborodov, V. L., Selivanova, I. A., and Terekhov, R. P. (2020). "ABTS/PP decolorization assay of antioxidant capacity reaction pathways," *International Journal of Molecular Sciences* 21(3), article no. 1131. DOI: 10.3390/ijms21031131

Kadam, P., Patil, M., and Yadav, K. (2018). "A review on phytopharmacopial potential of *Epilobium angustifolium*," *Pharmacognosy Journal* 10(6), 1076-1078. DOI: 10. 10.5530/pj.2018.6.181

Kiss, A. K., Bazylko, A., Filipek, A., Granica, S., Jaszewska, E., Kiarszys, U., Kosmider, A., and Piwowarski, J. (2011). "Oenothein B's contribution to the anti-inflammatory and antioxidant activity of *Epilobium* sp," *Phytomedicine* 18(7), 557-560. DOI: 10.1016/j.phymed.2010.10.016

Li, H., Liu, Z. H., Meng, X. X., Hua, R., Yang, X. Y., Li, Q., and Bi, K. S. (2021). "Study on the determination method and antioxidant activity of the multi-component contents of the classical prescription Qingzao Jiufei decoction," *Acta Pharmaceutica Sinica* 56(12), 3511-3517. DOI: 10.16438/j.0513-4870.2021-0680

Li, W., Zhang, X. Y., Chen, R., Miao, J. Y., Chen, Y. J., and Cao, Y. (2020). "Research progress on Oenothein B and its biological activity," *Science and Technology of Food Industry* 41(11), 348-352+368. DOI: 10.13386/j.issn1002-0306.2020.11.054

Maruška, A., Ugenskienė, R., Raulinaitytė, D., Juozaitytė, E., Kaškonienė, E., Drevinskas, T., Stelmakienė, A., Akuneca, I., Makaravičius, T., Tiso, N., Bartkuvienė, V., Kornyšova, O., Ragažinskienė, O., Ramanauskienė, K., and Briedis, V. (2017). "Analysis of antiproliferative effect of Chamerion angustifolium water extract and its fractions on several breast cancer cell lines," *Advances in Medical Sciences* 62(1), 158-164. DOI: 10.1016/j.advms.2016.08.002

Mu, L. (2021). "The annual output of *Eucalyptus* wood in Guangxi exceeded 30 million m^3 in 2020," *China Wood-Based Panels* 28(03), 47-48.

Okuyama, S., Furukawa, Y., Yoshimura, M., Amakura, Y., Nakajima, M., and Yoshida, T. (2021). "Oenothein B, A bioactive ellagitannin, activates the extracellular signal-regulated kinase 2 signaling pathway in the mouse brain," *Plants* 10(5), 1030. DOI: 10.3390/plants10051030

Pei, X. D., Xiao, J. S., Wei, G. J., Zhang, Y. M., Lin, F., Xiong, Z. C., Lu, L., Wang, X. L., Pang, F. G., Jiang, Y., and Jiang, L. H. (2018). "Oenothein B inhibits human non-small cell lung cancer A549 cell proliferation by ROS-mediated PI3K/Akt/NF-κB signaling pathway," *Chemico Biological Interactions* 298(16), 112-120. DOI: 10.1016/j.cbi.2018.09.021

Qi, S. X. (2006). "Introduction of *Eucalyptus* in china and its development status," *Guangxi Forestry Science* 35(04), 250-252. DOI: 10.3969/j.issn.1006-1126.2006.04.016

Ramstead, A. G., Schepetkin, I. A., Quinn, M. T., and Jutila, M. A. (2012). "Oenothein B, a cyclic dimeric ellagitannin isolated from *Epilobium angustifolium*, enhances IFNγ production by lymphocytes," *PLoS One* 7(11), article no. e50546. DOI: 10.1371/journal.pone.0050546

Santos, S. C., Carvalho, A. G., Fortes, G. A. C., Ferri, P. H., and Oliveira, A. E. (2014). "Variable-temperature NMR and conformational analysis of Oenothein B," *Journal of the Brazilian Chemical Society* 25(2), 282-289. DOI: 10.5935/0103-5053.20130295

Satoru, T., Yang, G. M., Teruaki, K., Yoshiharu, M., Yasumasa, K., Tomikazu, K., and Nobutoshi, M. (2019). "Oenothein B, dimeric hydrolysable tannin inhibiting HCV invasion from *Oenothera erythrosepala*," *Journal of Natural Medicines* 73(1), 67-75. DOI: 10.1007/s11418-018-1239-1

Schepetkin, I. A., Kirpotina, L. N., Jakiw, L., Khlebnikov, A. I., Blaskovich, C. L., Jutila, M. A., Quinn, M. T. (2009). "Immunomodulatory activity of Oenothein B isolated from *Epilobium angustifolium*," *The Journal of Immunology* 183(10), 6754-6766. DOI: 10.4049/jimmunoTangl.0901827

Sharma, A., Shahzad, B., Rehman, A., Bhardwaj, R., Landi, M., and Zheng, B. (2019). "Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress," *Molecules* 24(13), 2452. DOI: 10.3390/molecules24132452

Toth, B. H., Blazics, B., and Kery, A. (2009). "Polyphenol composition and antioxidant capacity of *Epilobium* species," *Journal of Pharmaceutical and Biomedical Analysis* 49(1), 26-31. DOI: CNKI:SUN:SSPJ.0.2013-08-069

Wang, J., Bai, X. M., Meng, X., and Jing, L. (2021). "Study on ultrasonic-assisted extraction process of tea polyphenols from Sichuan summer and autumn green tea," *Light Industry Science and Technology* 37(03), 1-4. DOI: 10.1016/j.jpba.2008.09.047

Xie, Y. J. (2019). *South Land – A Green Landscape with Treasure*, Party School of the Central Committee Press C *Eucalyptus* PC, Beijing, China.

Yoshida, T., Yoshimura, M., and Amakura, Y. (2018). "Chemical and biological significance of Oenothein B and related ellagitannin oligomers with macrocyclic structure," *Molecules* 23(3), 552. DOI: 10.3390/molecules23030552

Zhang, Q. (2021). "Extraction, purification and antioxidative activity of polysaccharides from yellow tea," *Food Research and Development* 42(14), 49-55. DOI: 10.12161/j.issn.1005-6521.2021.14.009

Article submitted: July 1, 2022; Peer review completed: August 21, 2022; Revisions accepted: September 6, 2022; Published: September 14, 2022.
DOI: 10.15376/biores.17.4.6094-6107