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Optimization of Hydrodistillation Extraction by Response Surface Methodology and Evaluation of the Antioxidant and Antimicrobial Activities of Essential Oils from Phragmites communis (Cav) Trin

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Abstract: The principal objectives of this study encompass three distinct aspects: firstly, to streamline the extraction process of *Phragmites communis* (Cav) Trin essential oil; secondly, to systematically analyze the essential oil's chemical composition; and finally, to evaluate biological activity. Through the system optimization experiment, the optimal extraction process was determined the crushing size was set to 10 mesh, the total solid-liquid ratio was 19:1 (mL/g), and the extraction time limit was 5 hours. Under this condition, the maximum yield of essential oil was 0.233% (mass fraction). In addition, the GC-MS (gas chromatography-mass spectrometry) composition of pure essential reed oil (PCT-Eos) was analysed, and 27 different compounds were detected. The major components included: 2-(2-ethylhexoxycarbonyl) benzoic acid (15.78%), 2,2'-methylenebis (6-tert-butyl- 4-methylphenol) (9.96%), pentacosane (4.97%), triacontane (4.96%), dibutyl phthalate (4.92%), dotriacontane (4.64%), and nonacosane (4.17%). The findings of the activity test demonstrated that this essential oil possessed a moderate antioxidant capacity, with IC_{50}/EC_{50} values of 2.42 ± 0.311, 3.5 ± 0.306, 6.5 ± 0.395, and 9.8 ± 0.557 mg/mL. In both microdilution assessments, concurrently exhibiting significant inhibitory effects on various pathogenic bacteria, the respective minimum inhibitory concentrations (MICs) were determined to be 72.00, 72.00, 43.20, 9.33, and 120.00 mg/mL. These findings indicate that PCT-Eos possesses significant potential for applications in the food, pharmaceutical, and chemical sectors due to its notable antimicrobial and antioxidant properties.

Keywords: *Phragmites communis* (Cav) Trin., Essential Oils, Hydrodistillation Extraction, Antimicrobial Activity, Antioxidant Activity

Introduction

Phragmites communis (Cav) Trin. (PCT) is a gramineous plant that thrives in aquatic or wet environments. As an economically significant crop in China, PCT has an annual production of approximately 3 million tons and serves various purposes. Large areas of PCT can conserve water sources and create healthy wetland ecosystems. The numerous roots of PCT also reinforce embankments. Air-conducting tissues present in the leaves stems, and rhizomes of PCT enable it to purify sewage and inhibit the growth of blue-green algae (Ding et al., 2018; Li & Hong, 2004; Li et al., 2007; Men and Hu, 2007). Given its high biomass, the entire PCT plant can be used as ideal feed for large livestock such as horses and cattle. It can also be dried and processed into hay and silage with a pleasant scent. PCT has been shown to enhance immune function in animals due to its content of flavonoids, polysaccharides, and other active substances. Incorporating reed into animal feed improves feed utilization and promotes positive growth in animals (Li et al., 2021). The "Compendium of Materia Medica" and the "Chinese Dictionary of Traditional Chinese Medicine" highlight various medicinal properties of PCT (Li, 2004; Miao et al., 2017). For instance, burning its flowers and blowing the smoke into the nose can stop bleeding and treat menstrual disorders. Boiled roots can help to restore liver function and alleviate liver dysfunction, treat hepatitis. Its leaves have the ability to clear the lungs, stop vomiting, treat pulmonary dysfunction, cholera-induced vomiting, gastric abscesses, choking, vomiting, diarrhea, stomach heat vomiting, and terminal dysentery. PCT products also offer excellent health benefits for patients with hypertension, hyperlipidemia, and diabetes (Wu et al., 2012).

Numerous studies have demonstrated that flavonoids, polyphenols, and sugars isolated from reeds possess antioxidant, free radical scavenging, antibacterial, and anti-cancer effects. Dai et al., (1994) identified six structurally similar flavonoid glycosides in PCT leaves. This was achieved by means of HPLC（high-performance liquid chromatography. Sun et al. (2011) reported that PCT leaves mainly contain ten flavonoid components, including apigenin, cirsiumin, and rutin. Miao et al., (2004) found that PCT can remove free radicals and exhibit anti-aging effects through the activity of specific enzymes. The high efficiency antioxidant effect of PCT extract was demonstrated by Yu et al. (2009) as well as Xu et al. (2010) in which the iodine value determination method was used for the correlation analysis. Furthermore, the study revealed that the antioxidative efficacy of the PCT extract is contingent its concentration level. Li et al. (2009) confirmed that the higher flavonoid content in PCT, the stronger its ability to inhibit the oxidation of lard. PCT roots also contain various acids, such as hydroxybenzaldehyde, pinadialdehyde, angelic acid, vanillic acid, and hydroxybenzoic acid (Ling et al., 2008). Among these, angelic acid can significantly reduce estrogen production, inhibits blood clot formation, and exhibits some antagonistic effects on tumors.

Plant essential oils are secondary metabolites that provide special fragrances and to have an important capability as antibacterial and antioxidant agents. PCT's robust essential oils enhance the appetizing quality of animals feed and potentially extended the shelf life of PCT-derived products. However, this phenomenon remains underexplored in existing scientific literature. In the present study, fresh PCT were used as raw material, and steam distillation was employed to extract essential oils (PCT-Eos). In order to achieve this, the BBD（Box-Behnken experimental design）was utilised to improve the extraction technique and GC-MS（gas chromatography-mass spectrometry was selected to analyse a comprehensive analysis of the chemical constituents of reed essential oil. A systematic evaluation of the antioxidant and antibacterial efficacy of PCT-Eos was conducted, thereby elucidating its potential applications in the domains of medicine, animal husbandry and chemical and pharmaceutical engineering. The work provided an important theoretical basis for the comprehensive development and harnessing of reed resources.

Materials and Methods

Plant Materials, Strains and Chemical Reagents

Phragmites communis (Cav) Trin. (PCT) samples were collected from Songnen Plain (longitude 123.76308, latitude 44.30925) in northeast China in August 2021. The plant samples, bearing the Voucher number PCT-004, were stored in the Herbarium of School of Chemistry and Pharmaceutical Engineering, Jilin Institute of Chemical Technology, Jilin, China. After washing and drying at ambient temperature, the PCT samples were cut into sections measuring 1 to 2 cm in length for subsequent use. The microorganisms for this experiment were collected from China Microbial Preservation Network, http://www.bzwzw.com/index.php, the strains selected included the *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 29213, as well as *Candida albicans* CMCC (F) 98001 and *Bacillus pumilus* ATCC 700814. It is evident that all chemical reagents in the experiment are of analytical purity and meet the technical requirements of experimental analysis.

Extraction Procedure

Desiccated PCT was pulverized and passed through sieves of varying apertures. The essential oil (PCT-Eos) was extracted from 30 g of reed [*Phragmites communis* (Cav) Trin] by the Clevenger steam distillation method. Table 1 lists the different operating conditions of the single factor test.

Table 1. Extraction conditions of the single-element test

	Ratio of solid to liquid (g/mL)	Extraction time (h)	Particle size (mesh)
Series 1	1:10	3, 4, 5, 6, 7	10
Series 2	1:10, 1:20, 1:30, 1:40, 1:50	5	10
Series 3	1:20	5	0, 10, 20, 30, 40, 50

The amount of essential oils gained was given by the following formula:

$$Y_{PCT-Eos} = \frac{W_1}{W_2} \times 100\% \quad (1)$$

where $Y_{PCT-Eos}$ is the yield of PCT-Eos (w/w), W_1 means the mass of reed essential oil(g), and W_2 means the mass of raw reed(g), respectively.

Design of Experimental and Statistical Analysis

As described by Li et al., (2014); Kusuma and Mahfud (2015); Vidács et al. (2018), in this study, a three-factor experimental design, with the objective of determining the optimal ratio combination in each parameter of the essential oil extraction process (Table 2), including extraction time, liquid-to-solid ratio, and sample grain size. Experimental data were statistically analyzed using a Box-Behnken design (BBD) with RSM at 3 levels. Table 3 outlines the 17 experimental points designed, and the results were presented accordingly. The outcomes derived from central composite design regression analysis are in Table 4. A predictive correlation equation based on the quadratic polynomial model was formulatedutilize using the following equation to estimate the yield of essential oils:

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i<j}^{n} \beta_{ij} X_i X_j + \varepsilon \quad (2)$$

Herein, Y represnts the anticipated extraction yield of essential oils, β_0 denotes the constant factor, the linear part is βi, the quadratic part is βii and the magnitude of the interaction effect between the independent variables Xi and Xj is βij. ε represents the random experimental error.

GC-MS Analysis of the Chemistry of Essential Oils

In the frame of this study, the gas chromatography-mass spectrometry (GCMS-QP-2010 plus) instrument, manufactured by Shimadzu Corporation, was utilised for the separation and identification of the chemical components of essential oils. The analytical system is equipped with an Rxi-5MS fused quartz column. The specification parameters of the chromatographic column are as follows: column length 30 m, inner opening diameter 0.25 mm, and stationary phase film thickness 0.25 μm. During the experiment, ultra-high purity helium gas (purity ≥99.999%) was introduced as the transport medium. The gas flow rate was maintained at 1.0 mL/min, as was the split ratio, which was set at 1:10. The column temperature procedure is as follows: the first stage of the process is to set the temperature to 50°C, and then maintain this for a period of 3 min. This is to be followed by an increase in the temperature to 100°C at a rate of 50°C/min. The temperature was further escalated at 100℃/min to reach 200℃, and finally to 290℃, where it was maintained for 10 min. The operational parameters included a pressure of 117.6 kPa, an injector temperature of 280℃, and an interface temperature of 230℃. The total flow rate was 25.0 mL/min, with a chromatographic medium flow rate of 2.0 mL/min and a liner speed of 51.3 cm/s. The system was configured to purge at a rate of3.0 mL/min, and the temperature of the ion source was meticulously regulated to 200°C. The mass range of mass spectrometry scanning is set at 35-600 m/z. The essential oil's chemical components were accurately identified by comparison with the NIST standard mass spectrometry database and mass spectrometry analysis (Adams, 2007).

Table 2. Range and levels of independent factors

Factors	Code units	Coded variable level		
		(-1)	(0)	(1)
Extraction time (h)	A	4	5	6
Ratio of solide to liquid (g/mL)	B	1:10	1:20	1:30
Particle size of sample (mesh)	C	0	10	20

Table 3. The PCT-EOS extraction process is found to be improved by adopting the three-factor Box-Behnken design

Std	Run	A	B	C	Extraction yield (%)	
		Extraction time (h)	Ratio of solid to liquid (g/mL)	Particle size of sample (mesh)	Experimental	Predicted
6	1	6(1)	1:20(0)	0(-1)	0. 11	0.124
3	2	4(-1)	1:30(1)	10(0)	0. 09	0.094
7	3	4(-1)	1:20(0)	20(1)	0. 13	0.116
16	4	5(0)	1:20(0)	10(0)	0. 23	0.232
2	5	6(1)	1:10(-1)	10(0)	0. 14	0.136
10	6	5(0)	1:30(1)	0(-1)	0. 06	0.078
1	7	4(-1)	1:10(-1)	10(0)	0. 08	0.111
8	8	6(1)	1:20(0)	20(1)	0. 09	0.111
15	9	5(0)	1:20(0)	10(0)	0. 26	0.232
5	10	4(-1)	1:20(0)	0(-1)	0. 11	0.089
4	11	6(1)	1:30(1)	10(0)	0. 13	0.099
9	12	5(0)	1:10(-1)	0(-1)	0. 15	0.14
13	13	5(0)	1:20(0)	10(0)	0. 20	0.232
11	14	5(0)	1:10(-1)	20(1)	0. 13	0.113
12	15	5(0)	1:30(1)	20(1)	0. 11	0.12
14	16	5(0)	1:20(0)	10(0)	0. 23	0.232
17	17	5(0)	1:20(0)	10(0)	0. 24	0.232

Antioxidant activity test

Determination of the radical scavenging capacity of DPPH The aim of this study was to evaluate the antioxidant performance of reed essential oil (PCT-EOS). To this end, the experimental method established by Dong et al., (2015) was referred to, and the determination of DPPH radical scavenging activity was carried out. The specific operation is as follows: take 2 mL of specific concentration of essential oil ethanol solution and 2 mL of 0.1 mmol/L DPPH ethanol solution, the substance should then be placed on a vortex mixer and agitated for 30 s in order to ensure uniform mixing. The mixed solution must then be transferred to a light-proof condition and reacted at room temperature (25±1℃) for 30 min, and then determine the absorbance at 517 nm wavelength. VC (L-ascorbic acid) and BHT (Butylhydroxytoluene) were used as control groups, ethanol as negative controls, and a blank group containing only essential oil and ethanol was set up. All experiments were measured in parallel for three times and averaged to improve data reliability. Free radical clearance (RSA) was thereafter evaluated by means of the following calculation:

$$\text{DPPH radical scavenging activity}(\%) = \frac{A_0-(A_i-A_j)}{A_0} \times 100 \quad (3)$$

Within the formula, Ai denotes the value of the sample measurement (comprising VC or BHT), Aj is the measurement of the blank solution devoid of DPPH, and A0 is the measured value of the control group. The capacity of essential oils to act as antioxidants was evaluated by calculating the half maximal Inhibitory Concentration (IC50), which is defined as the sample dosage required to eliminate 50% of DPPH free radical molecules. It is evident that the smaller the IC50 value, the stronger the free radical scavenging efficiency of the test substance.

$ABTS^+$ Free Radical Scavenging Activity

The aim of the present investigation was to enhance and modify the analytical protocol established by Huang et al. (2010) in order to evaluate the $ABTS^+$ radical scavenging efficacy of essential oil samples. The specific operational procedure is outlined as follows: to commence, a concentration of 7 mmol/L ABTS solution is mixed with a concentration of 2.45 mmol/L potassium persulfate solution, ensuring an equilibrium of volumes is achieved. Thereafter, the $ABTS^+$ free radical scavenging solution is prepared by reaction at room temperature for 16 h away from light. The reserve solution was then diluted with ethanol to stabilise its absorption at 734 nm to within 0.700±0.005. 0.3 mL of reed essential oil samples with varying concentrations (5-50 mg/mL) are then added to 2.7 mL of $ABTS^+$ working liquid, after which the light absorption value at 734 nm is measured following a light shielding reaction at 30℃ for 5 min. The calculation of $ABTS^+$ free radical clearance is achieved through the utilisation of the formula that has been deduced is as follows:

$$\text{ABTS}^{+}\text{ scavenging activity (\%)} = \frac{A_0 - A_S}{A_0} \times 100 \tag{4}$$

In this formula, A_0 is defined as the value of the absorption without the addition of the sample, and A_S is the measured value after the addition of the sample. VC and BHT were included as standard controls and the half inhibitory concentration (IC_{50}, mg/mL) was calculated. Reduction power measurement experiment.

This study adapted the method reported by Harkat-Madouri et al. (2015) in order to establish an experimental protocol for determining the reducing ability of reed essential oil (PCT-Eos). The specific operational process is outlined as follows: initially, the essential oil sample was prepared at concentrations ranging from 5-30 mg/mL (equating to a gradient of 5 mg/mL). During the experiment, 2.5 mL of the essential oil sample underwent a preliminary amalgamation with a 1% potassium ferricyanide solution (w/v) and 0.2 mol/L of phosphate buffer solution (pH 6.6). Following thorough agitation of the mixed liquid, it is to be placed in a constant temperature water bath at 50°C for a period of 20 min. Subsequent to the completion of the reaction, 2.5 mL of a 10% trichloroacetic acid solution (w/v) should be added, and the mixture thoroughly agitated. Thereafter, the centrifuge the mixture at a force of 3000×g for a period of 10 min. In conclusion, 2.5 mL of the serum should be collected and added to 0.5 mL of 0.1% anhydrous ferric chloride ($FeCl_3$, w/v) and 2.5 mL of ultrapure water, respectively. The resulting mixture was then subjected to a spectrophotometric analysis at a wavelength of 700 nm. The resulting data were then compared with the spectrophotometric analysis results of ascorbic acid (VC) and butylhydroxytoluene (BHT). The experimental results were analyzed using linear regression, and the effective concentration required to achieve an absorbance of 0.5 units was designated as EC_{50}.

Hydroxyl Radical Scavenging Activity Test

The evaluation of hydroxyl radical scavenging rate was conducted following the methodology established by You et al. (2011). A series of samples was prepared by dissolving them in ethanol solutions at concentrations of 5.79, 10.13, 13.5, 20.25 and 40.5 mg mL^{-1}. For each sample, 2.0 mL of the sample solution was collected and combined with 1.0 mL of 1, 10-phenanthroline, and both were dissolved in PBS. Following this, 1.0 mL of 1.865 mmol L^{-1} $FeSO_4 \cdot 7H_2O$ solution was added to the mixture, which was then thoroughly mixed. Then, we introduced 1.0 mL of H_2O_2 solution (0.03% by volume) into the reaction mixture, then maintained the temperature at 37℃ for 1 h. The absorbance at 536 nm (denoted as A_s) was recorded. The blank control (A_b) and negative control (A_n) consisted of reaction systems without H_2O_2 and without sample, respectively. The active control included VC and BHT. The elimination rate of the hydroxyl radical was formulaic as follows:

$$\text{Hydroxyl radical scavenging activity (\%)} = \frac{A_s - A_n}{A_b - A_n} \times 100\% \tag{5}$$

Evaluation of Antimicrobial Activities

To assess the antibacterial activity of PCT-Eos, we determined the Minimum Inhibitory Concentration (MIC) against *B. subtilis*, *B. pumilus*, *E. coli* and *S. aureus*, were determined. PCT-Eos were dissolved in a final concentration of 20% (v/v) Dimethyl Sulfoxide (DMSO) and then diluted into a concentration gradient ranging from 1.21 to 200 mg/mL, with each subsequent concentration reduced by a factor of 0.6, in a 96-well plate. A microbial cell suspension (with a cell count of 10^8 cfu/mL) was added to each well. A positive control was established using chloramphenicol at concentrations spanning from 0.73 to 200 mg/mL, while a negative control contained 20% DMSO without essential oils. To ensure the reproducibility and reliability of the results, each assay was initiated in triplicate. The minimum effective inhibitory concentration for microbial growth of PCT-Eos is designated as MIC.

Statistical Analysis

All the anti-bacterial and antioxidant experiments in this study were performed with three replicates, and the data were averaged and expressed as mean ± standard error. The data processing was achieved by employing the single-factor ANOVA method, facilitated by IBM SPSS, and the Duncan's multiple comparison test was employed to statistically analyse the differences between groups. The significance level was set at $p<0.05$.

Table 4. The ANOVA test of the response surface was conducted using the dimensionality reduction quadratic model[a];

Source	Sum of Squares	df	Mean Square	F Value	*P*-value Prob > F	
Model	5.563×10^{-6}	9	6.181×10^{-7}	7.27	0.0080	significant
A[b]	4.500×10^{-8}	1	4.500×10^{-8}	0.53	0.4906	
B[b]	1.512×10^{-7}	1	1.512×10^{-7}	1.78	0.2242	
C[b]	1.125×10^{-8}	1	1.125×10^{-8}	0.13	0.7269	
AB	1.000×10^{-8}	1	1.000×10^{-8}	0.12	0.7418	
AC	4.000×10^{-8}	1	4.000×10^{-8}	0.47	0.5150	
BC	1.225×10^{-7}	1	1.225×10^{-7}	1.44	0.2692	
A^2	1.632×10^{-6}	1	1.632×10^{-6}	19.18	0.0032	
B^2	1.503×10^{-6}	1	1.503×10^{-6}	17.67	0.0040	
C^2	1.503×10^{-6}	1	1.503×10^{-6}	17.67	0.0040	
Residual	5.955×10^{-7}	7	8.507×10^{-8}			
Lack of Fit	4.075×10^{-7}	3	1.358×10^{-7}	2.89	0.1659	not significant
Pure Error	1.880×10^{-7}	4	4.700×10^{-8}			
Cor Total	6.159×10^{-6}	16				
R^2	0.9033					
Adj R^2	0.7790					
Adeq Precision	6.907					

Notes:
[a] Results were obtained with Design Expert 8.0.6
[b] A: Extraction time (h); B: Ratio of solid to liquid (g/mL); C: Sample grain size (mesh)

Results

Univariate Experimental Analysis of Distillation Extraction of Essential Oil

The steam distillation extraction method was selected for this experiment due to its ease of operation, simplicity of equipment, low cost and minimal environmental impact. To optimize the extraction process, a factorial experimental design was employed, focusing on three single factors: extraction time, liquid-to-material ratio and sample grain size.

Relationship Between Extraction Time and Yield of Essential Oils Obtained from PCT

As you can see in Fig. (1A), the extraction efficiency of reed (PCT) essential oil is demonstrably correlated with the water distillation time. The findings of the research indicate that in instances where the distillation time is inadequate, there is a substantial decline in the yield of essential oils. However, with an increase in treatment duration, a marked increase in product yield was observed. The optimal extraction conditions, as indicated by the maximum oil yield, were attained when the distillation time was set at 5 h. This can be attributed to the incomplete extraction of essential oil within shorter period, as the gradual evaporation of essential oil components in PCT reaches completion around the 5 h mark. It has been proposed that when the distillation time exceeds 5 h, the heat sensitive components of the essential oil begin to degrade, resulting in a downward trend in the yield of the final product. This phenomenon may be attributed to the decline in the thermal stability of certain volatile compounds under prolonged high-temperature conditions. The experimental data demonstrate that an excessive prolongation of the extraction time not only fails to enhance the yield, but also results in a loss of active constituents.

Correlation Between Yield Efficiency of PCT-EOS and Solid-Liquid Ratio

The experimental data (Fig. 1B) provides clear evidence that adjusting the liquid-solid ratio can effectively regulate the output of PCT essential oil. A lower extraction yield is observed at a ratio of 1:10, likely due to insufficient submersion of PCT raw material, leading to gelatinization. The experimental data show that when the liquid-solid ratio rises to 20:1, the yield reaches its maximum value. Nevertheless, upon surpassing this critical value, the yield declines in proportion to the rise in the liquid-solid ratio. It has been claimed that increasing the material/liquid ratio promotes full contact between the water and the raw materials. It has been demonstrated through experimental means that an increase in the volume of the solvent can lead to an effective expansion of the solid-liquid contact area. It is evident that the yield of essential oils can be enhanced by virtue of two interrelated factors: firstly, the improvement of the mass transfer process, and secondly, the reduction of mass transfer resistance. (Kusuma and Mahfud, 2017). However, at higher ratios, such as 40:1 (v/w), the yield decreases. This decline could be due to the dissolution or potential chemical alterations of the oil components within the enlarged aqueous medium, as documented by Milojević et al. (2008).

Effect of Sample Grain Size of PCT on Essential Oil Extraction Rate

Figure (1C) shows the significant influence of the sample grain size on the quantity of essential oil extracted from PCT. An inverse relationship was observed between the sample grain size and the yield of essential oil. Large grain sizes led to reduced yields, while the peak yield was attained at a sample grain size equivalent to 10 mesh. When the sample grain size exceeds 10 mesh, the yield decreased because larger particles inhibited complete extraction of essential oils within a given time. Conversely, smaller sample grain sizes facilitate full contact between PCT and water vapor, enhancing internal extraction of essential oils and increasing yield (Zheljazkov et al., 2014). However, excessive milling can induce agglomeration and gelatinous transformation of the PCT powder, leading to uneven extraction conditions and inadequate yield, as corroborated by Kusuma and Mahfud (2017).

Optimization of Process Parameters for Hydrodistillation Extraction Based on RSM

In order to improve the extraction efficiency of reed (PCT) essential oil, the BBD (Box-Behnken experimental Design) method was adopted in this study to systematically optimize the key process parameters, this method involved three critical factors, each at three levels, to conduct a thorough response surface methodology analysis. A, B and C were defined as the observed variables, which were extraction time, the solid-liquid ratio and sample grain size, respectively, with the response value being the essential oil yield (Y). A comprehensive experimental setup comprising seventeen distinct sets was devised, with the details of factors and their levels tabulated in Table (2). Subsequently, Table (3) offers an extensive overview of the experimental design alongside the corresponding outcomes, whereas the results from the regression analysis on the central composite design are comprehensively presented in Table (4). The regression model demonstrates a significance, while $P<0.01$ indicating extreme significance and $P>0.05$ suggests its insignificance. The analysis highlights the substantial impact of A^2, B^2, and C^2 on the yield of essential oil, emphasizing their critical role in the process. The anticipated outcome, denoted as Y (w/w), can be mathematically predicted utilizing a quadratic polynomial equation, which is derived from a rigorous multiple regression analysis of the gathered experimental data:

$$Y = 0.23 + 0.008A - 0.014B + 0.004C - 0.005AB - 0.010AC + 0.017BC - 0.062A^2 - 0.060B^2 - 0.060C^2 \quad (6)$$

In the aforementioned equation, the code values A, B, and C represent the extraction time, the solid-liquid ratio, and sample grain size, respectively.

Seeking to verify the accuracy of the predictions made by the regression model, the parameters of the model were statistically analysed, we utilized the F-test and P-value as key assessment criteria. It can be known from the data in Table (4) that the combined parameters of F = 7.27 and P = 0.008 indicate that the established regression equation can not only accurately reflect the experimental data, but also pass the strict significance test. (Cheng et al., 2017). As demonstrated in Table (4), the model has excellent goodness of fit, with the coefficient of determination (R^2) reaching 0.90 and the corrected coefficient of determination at 0.78. The values mentioned previously encapsulate the overall variation observed in the model's response, whereas the precision of the model is evaluated through the determination of its coefficients. As depicted in Fig. (2), the standardized residuals fall within the ±3 range, confirming that the model's capacity to accurately fit the experimental data without any apparent outliers or anomalies (Hasni et al., 2017). A signal-to-noise ratio of 6.91 exemplifies the model's precision, surpassing the threshold of 4, which further validates the model's adequacy (Siatis et al., 2006). The F-value of 2.89 suggests that the variance in the fitting does not significantly differ from the pure error, indicating a proper model fit to the data. Table (4) reveals that the quadratic coefficients (A^2, B^2, and C^2) have been shown to exert a significant regulatory effect on the extraction rate of essential oil, with $P<0.05$, whereas the interaction coefficients (AB, AC, and BC) do not reach statistical, as evidenced by $P>0.05$.

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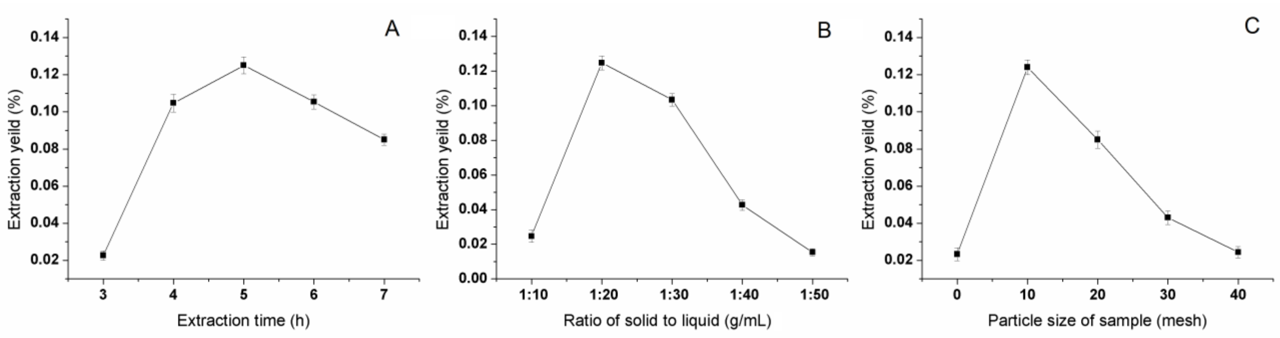

Fig. 1. The outcomes of single factor tests

A. Extraction time (s); **B.** Ratio of liquid to solid (mL/g); **C.** Sample grain size of sample (mesh).

The presented data were presented as mean ± SD (*n*=3).

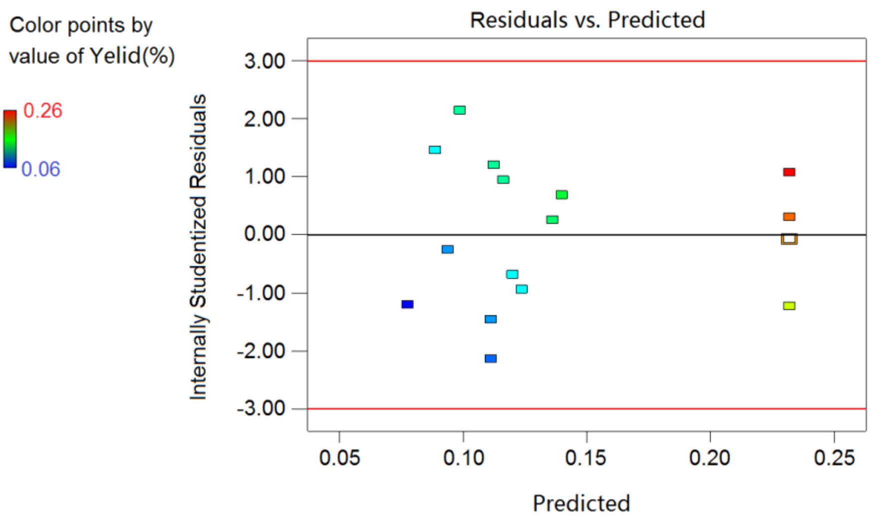

Fig. 2. Residuals and predicted values

The hierarchy of factors influencing PCT-Eos yield is led by the solid-liquid ratio (B), the extraction time (A), and the sample grain size (C). RSM visualizes the relationships between pairs of variables through three-dimensional surfaces and contour plots, as shown in Fig. (3-5), providing insights into the optimal conditions for each factor. The experimental data presented in Fig. (3) demonstrate that within a reasonable range, an increase in the solid-liquid ratio or extraction duration can effectively enhance the yield of essential oils. In Fig. (4), the synergistic effect of the yield of essential oil and sample grain size and extraction duration is graphically represented, showing a positive correlation that eventually reverses with further increases. Similarly, Fig. (5) illustrates that increasing both sample grain size and the solid-liquid ratio boosts the yield of essential oil up to a point, beyond which no significant improvement is observed. Experimental trials confirmed the accuracy of the model under optimal conditions: the key process parameters were determined by conducting a response surface analysis using Design-Expert 8.0.6.1 software. The duration of the extraction process was 5.06 h, the solid-liquid ratio was 18.83:1(mL/g), and a sample grain size of 10.08 mesh. Under these refined conditions, which were a time of 5 h, a solid-liquid ratio of 19:1 (mL/g), and a sample grain size of 10 mesh, the actual essential oil yield was 0.233% (w/w), closely matching the predicted yield of 0.226%. With only a 3.00% deviation, the results affirm the validity of the model predictions. In summary, the present study focuses on the refinement of the PCT-EOS extraction process through the application of the Response Surface Method (RSM), with the objective of achieving precise adjustments to critical indicators such as the extraction duration, the solid-liquid ratio, and the sample grain size has proven effective, ensuring a reliable approach for enhancing essential oil yield.

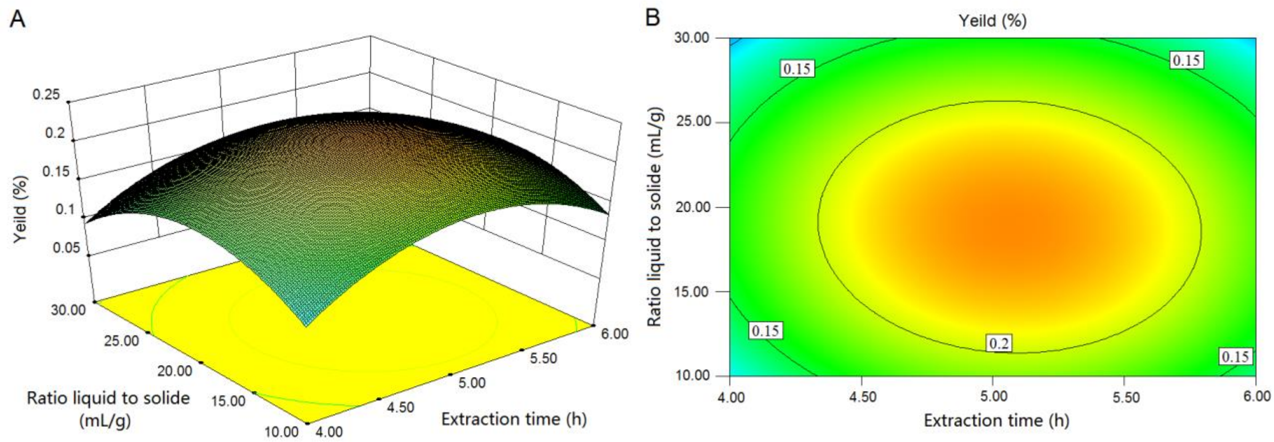

Fig. 3. The impacts of the solid-liquid ratio (mL/g) vs. extraction time (h) on the yield of Phragmites communis (Cav) Trin. essential oils. **A.** Response surface; **B.** Contour plots

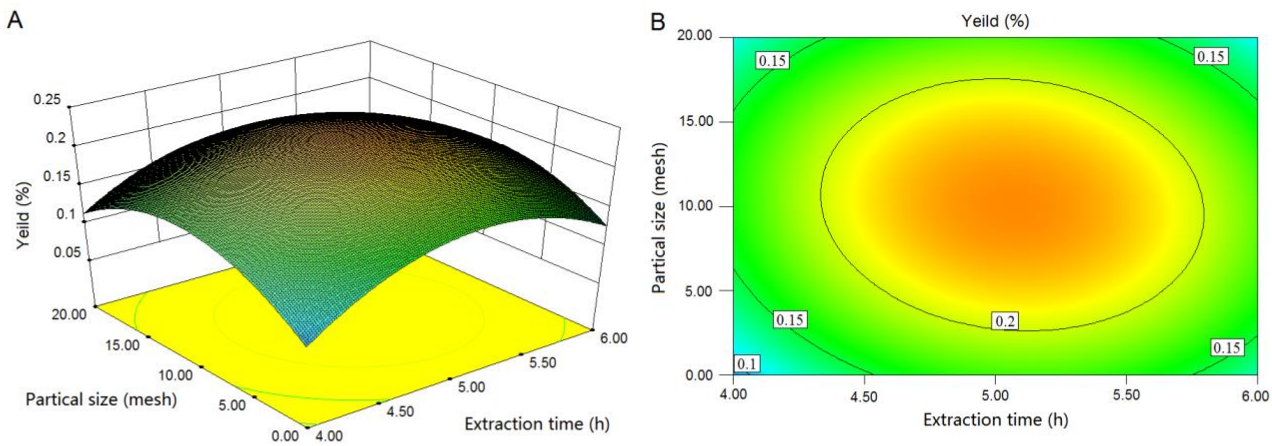

Fig. 4. The effects of sample grain size (mesh) vs. extraction time (h) on the yield of Phragmites communis (Cav) Trin. essential oils. **A.** Response surface; **B.** Contour plots

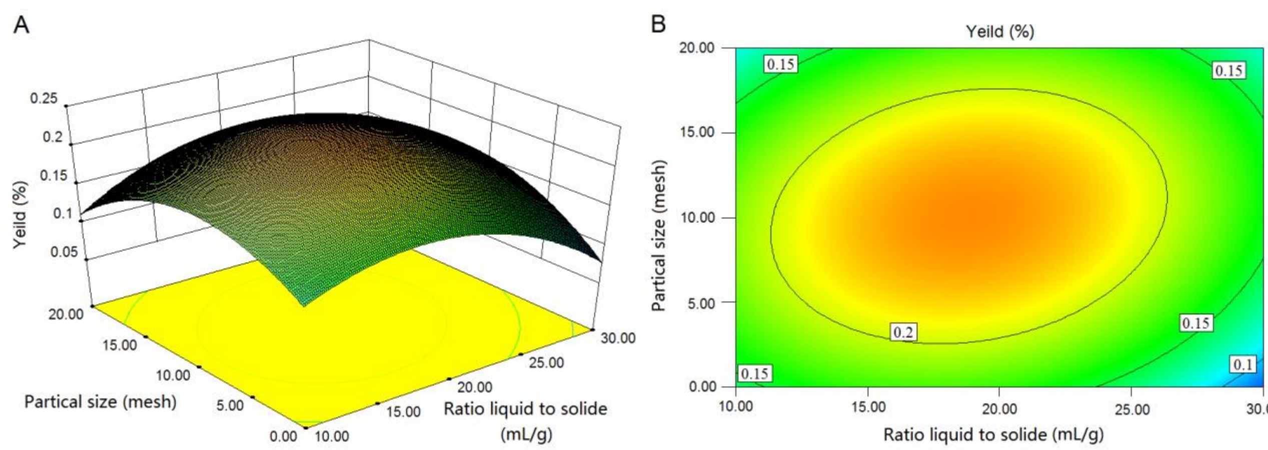

Fig. 5. The effects of sample grain size (mesh) vs. the solid-liquid ratio (mL/g) on the yield of Phragmites communis (Cav) Trin. essential oils. **A.** Response surface; **B.** Contour plots

Table 5. Relative percentage composition of PCT-Eos

Time (min)	CAS	Compounds	Formula	Area (%)	Type[b]	Saturability[a]
34.24	84-74-2	dibutyl phthalate	$C_{16}H_{22}O_4$	4.92	Est	U
36.586	29484-47-7	2-naphthalenemethanol, 1,2,3,4,4a,8a-hexahydro-α,α,4a,8-tetramethyl-, (2R,4aS,8aR)-	$C_{15}H_{24}O$	2.74	Alco	U
36.649	112-95-8	eicosane	$C_{20}H_{42}$	2.18	Alka	S
36.736	822-23-1	octadecyl acetate	$C_{20}H_{40}O_2$	2.24	Est	S
36.955	629-94-7	heneicosane	$C_{21}H_{44}$	2.10	Alka	S
37.351	19781-73-8	n-heptadecylcyclohexane	$C_{23}H_{46}$	2.14	Alka	S
37.445	646-31-1	tetracosane	$C_{24}H_{50}$	3.28	Alka	S
37.736	4209-22-7	1-bromotriacontane	$C_{30}H_{61}Br$	2.52	Alka	S
38.218	629-99-2	pentacosane	$C_{25}H_{52}$	4.97	Alka	S
38.548	119-47-1	2,2'-methylenebis(6-tert-butyl-4-methylphenol)	$C_{23}H_{32}O_2$	9.96	Alco	U
38.807	6703-81-7	11-cyclopentylhenicosane	$C_{26}H_{52}$	2.54	Alka	S
38.926	112-92-5	octadecanol	$C_{18}H_{38}O$	2.17	Alco	S
39.024	630-03-5	nonacosane	$C_{29}H_{60}$	4.17	Alka	S
39.15	63307-60-8	4-(2,6-dimethylcyclohexyl)-2-butanone	$C_{12}H_{22}O$	2.44	Ket	S
39.323	638-68-6	triacontane	$C_{30}H_{62}$	4.96	Alka	S
39.57	4376-20-9	2-(2-ethylhexoxycarbonyl)benzoic acid	$C_{16}H_{22}O_4$	15.78	Est	U
39.665	62108-50-3	1-bromotetratriacontane	$C_{34}H_{69}Br$	2.60	Alka	S
39.918	544-85-4	dotriacontane	$C_{32}H_{66}$	4.64	Alka	S
40.248	14167-59-0	tetratriacontane	$C_{34}H_{70}$	3.03	Alka	S
40.503	630-06-8	hexatriacontane	$C_{36}H_{74}$	3.95	Alka	S
41.02	16507-61-2	oleyl chloride	$C_{18}H_{35}Cl$	2.12	Alke	U
42.133	4181-95-7	tetracontane	$C_{40}H_{82}$	2.90	Alka	S
42.823	57397-30-5	labdane-6β,13-diol	$C_{20}H_{38}O_2$	2.18	Alco	S
43.581	7098-22-8	tetratetracontane	$C_{44}H_{90}$	2.54	Alka	S
44.314	468-84-8	4a,7,7,10a-tetramethyl-2,5,6,6a,8,9,10,10b-octahydro-1H-benzo[f]chromen-3-one	$C_{17}H_{28}O_2$	2.82	Est	S
46.939	153650-82-9	2,6,10,15,19,23-hexamethyl-2,6,14,18,22-tetracosapentaene-10,11-diol	$C_{30}H_{52}O_2$	2.06	Alco	U
49.655	93175-76-9	15-isobutyl-(13.alpha.H)-isocopalane	$C_{14}H_{22}O$	2.05	Ket	U

Notes:
[a] S= Saturated groups, U=Unsaturated groups
[b] Est: Ester; Alco: Alcohol; Alka: Alkane; Ket: Ketone; Alke: Alkene

The Essential Oil Obtained from the PCT were Determined by GC-MS

The mass spectra were acquired using a GC-MS system for analysis. The calculation of the relative content of each chemical was carried out using the peak area normalisation method. As Table (5) shows, a maximum of 27 different compounds were identified in reed essential oil. The composition analysis demonstrated that these volatile components could be classified into two categories: unsaturated and saturated, accounting for 39.63 and 60.37%, respectively. The unsaturated components comprised alcohols (14.76%), alkenes (2.12%), esters (20.7%), and ketones (2.05%). Conversely, the saturated constituents consisted of alcohols (4.35%), alkanes (48.52%), ethers (5.06%), and ketones (2.44%). The major components were 2-(2-ethylhexoxycarbonyl) benzoic acid (15.78%), 2,2'-methylenebis(6-tert-butyl-4-methylphenol) (9.96%), pentacosane (4.97%), triacontane (4.96%), dibutyl phthalate (4.92%), dotriacontane (4.64%), and nonacosane (4.17%. Together, these major components constituted 49.4% of the total essential oil content. Additionally, 15 types of C_{20}-C_{35} n-alkanes represented approximately 20% of the total composition, potentially linked to the wax coating on the surface of the reeds (Talik et al., 2018). It is noteworthy that AO2246, also known as 2,2'-methylene bis (6-tert-butyl-4-methylphenol), was detected in the PCT-Eos system. This phenolic antioxidant is extensively utilized in various applications, including natural rubber, synthetic rubber, latex, petroleum products, and diverse food packaging materials and personal care products, such as cosmetics. The product under discussion has been demonstrated to have significant application value in the field of industrial plastics. This is due to its low toxicity, low pollution, good thermal stability and good antioxidant activity and so on. This observation may be attributed to the reed's capacity to isolate pollutants, as evidenced by the recorded wastewater discharge levels of 0.00068 μg/L, the dry weight (dw) of 1.7 to 3.53 ng/g for sludge samples in China, and the dry weight (dw) of 0.39 ng/g for house dust collected in North America. (Liu et al., 2015a-b; Wu et al., 2020; Yu and Lin, 2023). Furthermore, the identification of halogenated compounds such as 1-bromotriacontane and oleyl chloride suggests a possible connection to the water purification capabilities of the reed, highlighting its potential in environmental remediation efforts.

Antioxidant Activity

The biological potency of essential oils is predominantly evaluated through their antioxidant activity, a critical metric that underscores their potential health benefits. Plant essential oils are rich mixtures of many functional groups of changing polarity, and their chemical structure is complex and diverse. The structural characteristics of essential oils are the key factor determining their diverse chemical and physical profiles. Research has shown that these natural substances generally manifest antioxidant activity, which can be broadly classified into direct and indirect categories. Under normal physiological conditions, the body maintains a dynamic equilibrium of free radicals, which play roles in cellular processes such as proliferation, differentiation, metabolism, and the repair of damaged molecules. The balance of these processes relies on the body's intrinsic antioxidant defense mechanisms. Phenolic compounds found in plant essential oils exhibit high reactivity with free peroxides, thereby neutralizing or reducing their activity (Yang et al., 2024). An in-depth study of the antioxidant properties of PCT-Eos, a multifaceted approach was adopted, incorporating four methods: DPPH, $ABTS^+$, reducing ability and hydroxyl radical scavenging. Each method offers unique insights into different aspects of antioxidant activity, and the results were derived from triplicate experiments, with VC and BHT serving as benchmark positive and negative controls.

The Diphenyl picryl hydrazinyl radical (DPPH), renowned for its stability, acts as a primary monitoring agent for in vitro antioxidant evaluations. This is a typical example used for the evaluation of antioxidants in *vitro*. A concentration-response relationship within the 1.6-3.2 mg/mL range was observed, where increasing sample concentration correlated with enhanced DPPH-scavenging activity (Fig. 6A). The present study systematically investigated and examined the antioxidant properties of reed (PCT) essential oil. While antioxidant properties have been documented in various plant-derived essential oils (Alimpić et al., 2015; Wang et al., 2015; Ud-Daula et al., 2016), this investigation represents an inaugural assessment for PCT-Eos. Compared to the benchmarks VC (IC_{50} of 0.68±0.023 μg/mL) and BHT (IC_{50} of 0.13±0.003 μg/mL), the essential oils exhibited reduced scavenging efficacy, as evidenced by their higher IC_{50} value of 2.42±0.311 mg/mL (Table 6).

A substantial body of peer reviewed literature has confirmed that the $ABTS^+$ free radical scavenging method has become one of the standard methods for the bioassay of the essential oils extracted from plants have antioxidant properties, which can accurately evaluate their antioxidant properties and has been commonly cited in the field of natural product bioactivity evaluation. (Ud-Daula et al., 2016; Alimpić et al., 2015; Wang et al., 2015). This assay boasts the advantage of being adaptable to a broad array of compounds, allowing for an inclusive assessment of their potential antioxidant properties. Additionally, the aromatic oil samples were determined by the $ABTS^+$ free radical scavenging assay, was observed to vary in accordance with their concentration, as depicted in Fig. (6B). However, when benchmarked against well-established antioxidants such as ascorbic acid BHT (IC_{50} 0.01±0.003 μg/mL) and VC (IC_{50} 1.02±0.029 μg/mL), the essential oil in question exhibited a substantially elevate IC_{50} value of 3.5±0.306 mg/mL, as tabulated in Table (6).

Figure (6C) illustrates the reducing capacity of the essential oils sample, demonstrating that the test oil is effectively facilitates the reduction of Fe^{3+} to Fe^{2+}. The date suggests a dose-dependent relationship with respect to the enhancing power of the oil's reducing activity. Upon evaluation, it was discerned that reducing capability of BHT (EC_{50} 0.12±0.002 μg/mL) and VC (EC_{50} 0.03±0.003s μg/mL) markedly lagged behind that of the experimental oil, which demonstrated an EC_{50} of 6.5±0.395 mg/mL (Table 6).

Hydroxyl radicals, as a type of reactive oxygen species, are known for their potential to induce damage to various cellular components, including erythrocytes, DNA, cell membranes, and polysaccharide. Given their destructive capability, the scavenging activity against hydroxyl radicals serves as a critical indicator for assessing the antioxidant capacity of substances. As illustrated in Fig. (6D) and Table (6), the IC_{50} values of PCT-Eos 9.8±0.557 mg/mL and VC against hydroxyl radicals were found to be 39.55±4.94 mg/mL and 492.27±19.96 µg/mL, severally.

This work systematically elucidates the anti-oxidative properties of reed essential oil (PCT-Eos) for the first time, a finding that echoes the conclusions of Cui et al. (2018) study on the biological activity of plant essential oils. The results of the experimental data demonstrate that the synergistic effect of multiple active components in PCT-Eos is the key reason for its antioxidant activity, which collectively contribute to the overall biological activity through complex interactions. Of particular interest is the presence of 2-(2-ethylhexoxycarbonyl) benzoic acid, a compound typically used as a plasticizer but also identified within the essential oils of certain plants, where it contributes to their antioxidant activity. Another notable component is 2,2'-Methylenebis(6-tert-butyl-4-methylphenol), also known as AO2246. This low-molecular-weight synthetic phenolic antioxidant has garnered recognition for its potent antioxidant capabilities, as reported by Liu et al., (2015a). Regarding natural sources, the hexane extracts of *Lycium barbarum* L. revealed pentacosane as the predominant constituent (17.74%), displaying potent antioxidant activity with an IC_{50} of 5.74 µg/mL against DPPH free radicals, nearly equivalent to that of VC (Alaa Nasser and Ahlam Hussein, 2021). Conversely, the methanol extrace of *Tithonia diversifolia* leaf, enriched with pentacosane (22.01%), manifested antioxidant scavenging properties, albeit with a higher IC_{50} value of 120.264 µg/mL (Roopa et al., 2021). The hexane extract of *Dendrobium crepidatum*, contained tetracosane, heneicosane, tetratriacontane, hexatriacontane, and triacontane accounted of 16.64% totally, exhibited antioxidant activity of DPPH free radicals scavenged with IC_{50} 306.77±51.14 µg/mL (Paudel et al., 2019). The primary constituent of the ethyl acetate extract derived from *Avicennia officinalis* L. is dibutyl phthalate. Notably, this crude extract exhibited promising antioxidant properties, as evidence by its IC_{50} values of 23.60±0.43 µg/mL, determined through the DPPH assay methodology (Lalitha et al., 2021). These comparisons underscore the diversity and complexity of natural products and highlight the potential of PCT-Eos as a novel source of antioxidants, warranting further investigation into its composition and mechanism of action.

It cannot be avoided that the antioxidant activity of the PCT-Eos was weaker than that of the positive controls VC and BHT, which may be caused by the complex composition in the PCT-Eos and the lower concentration of the active components. The study by El-Massry et al. (2002), the efficacy of a mixture's antioxidant activity is not solely determined by the concentration of its constituent compounds but also significantly influenced by the molecular structure of these compounds and their potential interactions. The properties of essential oils pertaining to antioxidation and scavenging of free radicals exhibit a relatively diminished level, potentially stemming from the extended timeframe necessary for their extraction. Notably, the prolonged and temperature-intensive extraction process leads to the breakdown, hydrolysis, and solubilization of bioactive constituents and heat-sensitive elements within the boiling medium (Djeridane et al., 2006; López et al., 2007). These factors can compromise the integrity and effectiveness of the extracted compounds, thereby affecting the overall antioxidant capacity of the final product. Thus, while PCT-Eos demonstrates antioxidant potential, its efficacy may be limited by both compositional complexity and the challenges associated with preserving the integrity of its bioactive components during extraction. Future research might focus on optimizing extraction techniques to better retain the potency of these components, potentially enhancing the antioxidant profile of PCT-Eos.

Table 6. Antioxidative capacities of PCT-Eos, VC and BHT

Samples	$ABTS^+$ (IC_{50})	DPPH (IC_{50})	Reducing power (EC_{50})	Hydroxyl radical (IC_{50})
PCT-Eos (mg/mL)	3.5±0.306	2.42±0.311	6.5±0.395	9.8±0.557
VC (µg/mL)	1.02±0.029	0.68±0.023	0.03±0.003	39.55±4.94
BHT (µg/mL)	0.01±0.003	0.13±0.003	0.12±0.002	492.27±19.96

[a] PCT-Eos: Phragmites communis (Cav) Trin. essential oils; VC: vitamin C; BHT: butylated hydroxytoluene

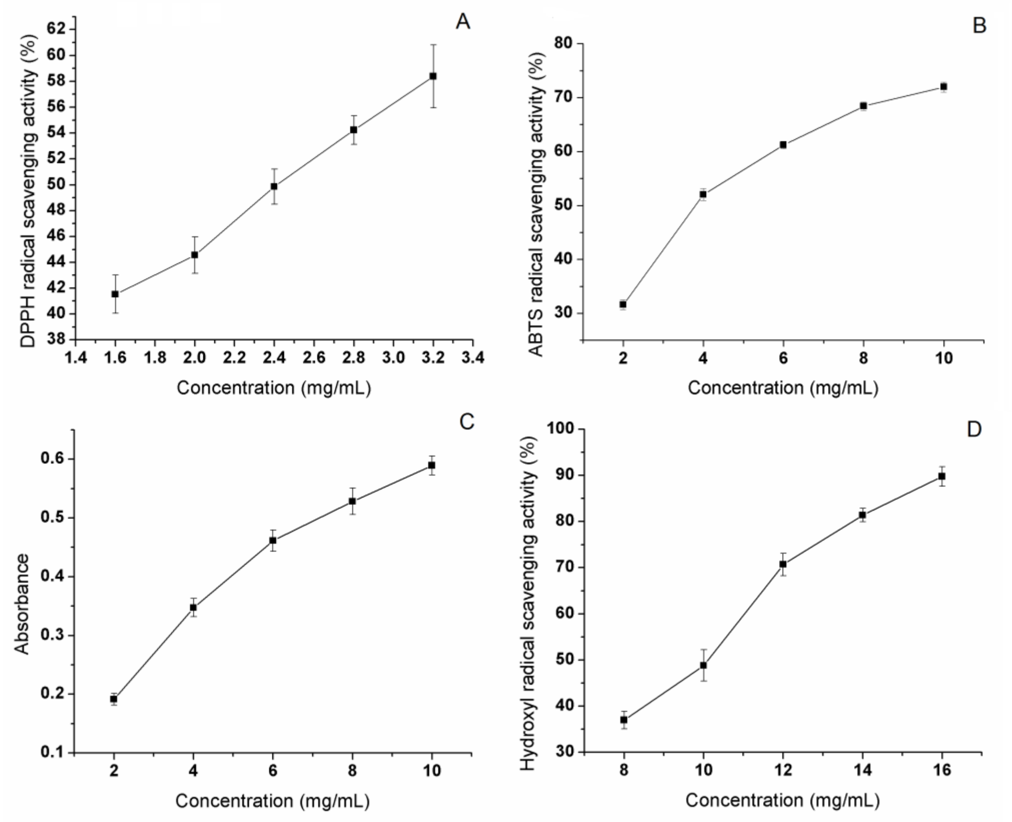

Fig. 6. Radical scavenging activity (%) of Phragmites communis (Cav) Trin. essential oils.
A. DPPH; **B.** ABTS; **C.** Reducing power; **D.** Hydroxyl radical

Antimicrobial Effect

Feng et al. (2001) confirmed the antibacterial effect of PCT through screening tests that evaluated 56 different plants for their antibacterial properties. Building on this foundation, the assessment of PCT-Eos' antimicrobial potential encompassed a comprehensive array of microbial targets, including three Gram-positive strains (G^+), a Gram-positive strain (G^-), *B. subtilis*, *E. coli* and *S. aureus*, and *B. pumilus* and a fungus, *C. albicans* (Table 7). The results were as follows: the MIC of the samples tested ranged from 9.33 to 120 mg/mL, indicating that PCT had modest antimicrobial activity against the above five microbes. Notably, PCT-Eos demonstrated its most potent activity against *E. coli*, with an MIC of 9.33 mg/mL. This heightened efficacy against *E. coli* may be attributed to the bacterium's particular sensitivity to PCT-Eos, possibly enhanced by the synergistic interactions among the constituent components within PCT-Eos. The principal component of PCT-Eos was identified as 2-(2-ethylhexoxycarbonyl) benzoic acid, a compound known for its antimicrobial properties (Rizwan et al., 2012). Notably, this compound was detected at a concentration of 40.30% in the ethanol of *Polygonum chinense* L., which demonstrated enhanced antimicrobial activity (Neelamegam and Ezhilan, 2012). Furthermore, the compound in question was discerned within the n-Hexane fraction extracted from *Azadirachta indica* A.Juss (Neem) leaves, demonstrating antifungal efficacy (Akpuaka et al., 2013). Importantly, the chloroform extract of *Ehretia serrata* leaves prominently featured 2,2'-Methylenebis(6-tert-butyl-4-methylphenol) as its primary component, the MIC values for this constituent ranged from 1.2 to 4.4 mg/mL against an extensive panel of 36 bacterial strains, it effectively inhibited the growth of the majority of these strains at a concentration of 3.6 mg/mL, as reported by Waheed et al. (2019). Add to that, the PCT-Eos also contains a certain amount of C_{20}-C_{44} aliphatic hydrocarbons (eicosane, heneicosane, tetracosane, pentacosane, nonacosane, triacontane, dotriacontane, tetratriacontane, hexatriacontane, tetracontane, and tetratetracontane), the total content of the aforementioned compounds was found to be 38.72%, which suggests that these substances may play a significant role in antibacterial functions. A comparable composition, dominated by aliphatic hydrocarbons (comprising 49.3% and exemplified by pentacosane, hexacosane, octacosane and heptacosane), has been observed in the essential oil of *Jatropha integerrima* Jacq seeds, this similarly supports the hypothesis that these hydrocarbons contribute to antimicrobial activity (Eshilokun et al., 2007). The specified essential oil exhibited inhibitory properties towards G^+ bacteria, specifically *Bacillus cereus* and *Staphylococcus aureus* (MIC ≤ 625 µg/mL). Nevertheless, its effectiveness was limited when confronted with the G^- bacteria, *Pseudomonas aeruginosa* (MIC=1250 µg/mL). The most topical study by Carev et al., (2023) points out that, ranging from 57.89 to 81.82%, of Centaurea-derived essential oils, enriched with pentacosane, displayed promising MICs of ≤ 500 µg/mL against a diverse panel of seven microbial species, encompassing *E. faecalis*, *E. coli*, *S. aureus*, *B. subtilis, P. aeruginosa*, *K. pneumoniae* and *C. albicans*. Furthermore, essential oils containing tetracosane and nonacosane were found to exhibit MICs within the ranges of 31.58-50% and 31.58-63.64%, respectively, highlighting their varied potency. In research conducted by Mini Shobi and Gowdu Viswanathan (2018), dibutyl phthalate, sourced from *Begonia malabarica*, was found to possess broad-spectrum antimicrobial potential. This compound exhibited activity across a concentration range from 6.25 mg/mL-100 mg/mL, effectively targeting microorganisms such as *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Escherichia coli*, *Micrococcus luteus*, *Vibrio cholerae, Klebsiella pneumoniae*, *Shigella flexneri* and *P. aeruginosa*. Notably, the dibutyl phthalate extracted from *H.* (*Soestella*) *caerulea* displayed potent antibacterial activity towards G^+ bacteria, including *Bacillus subtilis*, *Bacillus cereus*, and *Staphylococcus aureus*, with MIC of 500 µg/mL. Additionally, Shafeian et al. (2022) reported the minimum fungicidal concentration (MFC) of dibutyl phthalat against *Candida albicans* to be 1000 µg/mL, further underscoring its antimicrobial prowess. Li et al., (2023) reported that dibutyl phthalate markedly inhibits the proliferation of the fungus Fusarium. Upon treatment with this compound, observable deformations in the mycelial cells were noted, along with a marked inhibition of colony expansion and spore production. The literature extensively explores the antimicrobial properties of water-extracted plant-derived essential oils, although the mechanisms underlying these effects have not been entirely consistent. Agyekumwaa (2023) posited that the bactericidal and bacteriostatic properties of plant essential oils are predominantly attributable to their capacity to compromise the structural integrity of microbial cells. Notably, these natural products can affect the integrity of bacterial cell walls and membranes, causing leakage of cell contents and ultimately microbial death. Rhayour et al. (2003) suggested that essential oils inhibit bacteria growth by interfering with the biosynthesis of primary metabolites, such as nucleic acids and proteins. This interference can disrupt critical cellular processes necessary for bacterial survival. Additionally, a synergistic action involving multiple mechanisms may contribute to their efficacy. A plethora of body of research has shown that plant essential oils can compromise the structural integrity of bacteria, thereby compromising the integrity of their cell wall and the protein structure of their cell membrane. This process has been shown to affect the expression of bacterial genetic material, ultimately resulting in the eradication of bacteria and the exertion of an antibacterial effect. Simultaneously, phenolic compounds found in plant essential oils have been shown to recognize the key role in their antibacterial effect. These phenolic substances interact with the double-layered phospholipid structure of the cellular membrane of bacteria, increasing membrane acidity and permeability. This interaction disrupts essential cellular functions, ultimately leading to bacterial inhibition (Wang and Zhai, 2023; Wang et al., 2023). The increased permeability caused by phenolic compounds can result in the leakage of vital cellular components, thereby compromising the integrity and functionality of the bacterial cell. This mechanism underscores the significant contribution of phenolic compounds to the overall antibacterial efficacy of essential oils. For PCT-Eos, further research is necessary to fully understand and enhance its antibacterial mechanisms.

Table 7. The minimum inhibitory concentration of PCT-Eos[a] on the tested bacteria

	B. subtilis	S. aureus	E. coli	B. pumilus	C. albicans
PCT-Eos (mg/mL)	72.00	72.00	9.33	43.20	120.00
Chloramphenicol (mg/mL)	< 0.73	< 0.73	< 0.73	< 0.73	< 0.73

[a] PCT-Eos: Phragmites communis (Cav) Trin. essential oils

Conclusion

The extraction conditions of *Phragmites communis* (Cav) Trin. essential oils were optimized as below: an extraction time of 5 h, the solid-liquid ratio of 19:1 (mL/g), and a sample grain size of 10 mesh. Under such condition, the optimal yield of essential oil was determined to be 0.233% (w/w). The PCT-Eos was found to contain 27 components and demonstrated notable antimicrobial activity against 5 identified microorganisms, as well as appropriate efficacy in four antioxidant systems. The findings of this study lay a solid foundation for the further exploration of the untapped potential of PCT-Eos, with significant implications for the comprehensive development and utilization of reed resources across various fields, including feed, food, and other industries. Advancements in extraction technology and life sciences continue to drive the discovery of novel, environmentally friendly compounds. Natural plant-derived antimicrobial and antioxidative agents show considerable promise in replacing synthetic compounds. The prudent development of safe, practical, efficient and cost-effective bioactive substance from plants remains a prominent area of research.

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Ethics

This article is an original manuscript; all authors read and approved the final version of this manuscript. The authors declare their responsibility for any ethical issues that may arise after the publication of this manuscript.

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