Optimization of Extraction Process of Pine Needle Essential Oil by Response Surface Methodology and its Chemical Composition Analysis

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The extraction of essential oil from pine needles was optimized by response surface methodology, and the following optimal conditions were obtained: a fresh pine needle of 100 g, an extraction time of 2 h, a water dosage of 850 mL, and a NaCl concentration of 2.50%. The extraction yield of essential oil was 0.611% under optimal conditions, which was extremely close to the predicted value. The extraction yields of essential oil from needles of 12 common pines in Guangxi were compared. The contents of essential oil in needles of *Pinus massoniana, Pinus crassicorticea*, and *Pinus taeda* were relatively higher than other pines. A total of 44 chemical components were identified by GC-MS, including 12 monoterpenes, 14 sesquiterpenes, and 12 alcohols. The chemical components of essential oil from different pines have their own features, and it is speculated that they have good and diversified application potential in the fields of medicine, food, spices, and so on. The chemical compositions of essential oil with high extraction yield have similar characteristics. This phenomenon can be used as the basis and means for the selection of pines with high content of essential oil in needles.

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Keywords: Response surface methodology; Pine needles; Essential oil; Extraction yield; Chemical composition

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INTRODUCTION

Pines, a large group of plants (over 100 species), play an indispensable role in the global ecosystem (Gernandt *et al*. 2005; Zeb *et al*. 2019). They are widely distributed with large reserves around the world, and they have multi-directional economic value (Aikaterini *et al*. 2021). For example, pine wood acts as an important high-quality building material and pulp raw material (Moral *et al*. 2017; Wang *et al*. 2018). As a chemical raw material, oleoresin plays a significant role in industrial productions (Xie *et al*. 2019a,b; Shipra *et al*. 2021). Moreover, the pine fruit can be processed into delicious nuts and seeds for food. However, pine needles rarely have been investigated for their applications.

Pine needles possess a huge storage capacity, but their added value is relatively low, as they are used often as feed additives (Anderson 1985). Plant by-products are the source of natural bioactive compounds. In recent years, plant extracts have played an important

role in the fields of medicine, cosmetics, *etc*. (Sanja *et al*. 2008; Feyza *et al*. 2009; Hammami *et al*. 2016; Soltani *et al*. 2018; Peach *et al*. 2019; Chen *et al*. 2021). Therefore, it is necessary to investigate the extraction of essential oil from pine needles. It has been reported that pine needle essential oil (PNEO) has antibacterial, anti-inflammatory, and antioxidant effects (Chalchat *et al*. 1985; Dob *et al*. 2005a,b; Joseph 2017; Zeng *et al*. 2012). The published research on PNEO mainly focuses on its physiological and biochemical properties. There are few studies on the optimization and improvement of the extraction process of PNEO, the breeding of pine varieties with high content of PNEO, or the differences of chemical components of PNEO from different sources.

The common extraction methods of plant essential oil are as follows: steam distillation, microwave-assisted, organic solvent extraction, and supercritical extraction (Belhachat *et al*. 2018). Steam distillation is most suitable for PNEO extraction due to its simplicity, convenience, environmental protection, and low cost. To optimize this process, a single-variable method would require changing one variable and keeping other variables unchanged. The disadvantage of this method is that the influence of interaction among variables on the results is ignored. In this case, the number of experiments needs to be increased to obtain better results, which results in increased cost. Response surface methodology can solve these problems well because it can optimize the conditions of multivariable systems and reduce the number of experiments (Zermane *et al*. 2014).

In this paper, the steam distillation method was used to extract PNEO. The extraction yield of PNEO under different conditions (extraction time, water dosage and concentration of NaCl) was explored, and the extraction process was optimized by response surface methodology. The optimized extraction method was used to extract the PNEO from several common pines and their varieties in Guangxi. The differences of content of PNEO from different pines were compared. The chemical composition of PNEO was identified by GC-MS, and the characteristics of PNEO from different sources were analyzed. These studies provide data for the application and the structure-activity relationship of PNEO with different chemical composition and contents.

EXPERIMENTAL

Materials

The fresh needle samples used in the experiment were taken from the germplasm resource collection library of Nanning Institute of Forestry Science (23°10'N and108°00'E), Other chemicals and solvents were commercially available as standard laboratory-grade.

Extraction of PNEO

The fresh pine needles were broken with a high-speed agitator. A mixture of 100 g of broken sample and 800 mL of 2% sodium chloride aqueous solution was added to a 2000-mL single-necked flask with oil-water separator and condensing tube. The single-necked flask was placed in an electric heating sleeve for the extraction reaction. After extraction for 2 h, the upper oil was collected from the oil-water separator, and the PNEO was obtained after drying with anhydrous magnesium sulfate. The extraction yield was calculated as follows,

$$\text{Yield (\%)} = \frac{Mass\ of\ PNEO}{Mass\ of\ fresh\ needles} \times 100 \quad (1)$$

Single Factor Experimental Design

The effects of extraction time, water dosage, and concentration of NaCl on the extraction yield of PNEO were studied, and the test conditions shown in Table 1 were set.

Table 1. Factors and Levels in the Single-Factor Experiment

Factor	Level				
	1	2	3	4	5
A: Extraction time (h)	0.5	1.0	1.5	2.0	2.5
B: Water dosage (mL)	600	800	1000	1200	1400
C: Concentration of NaCl (%)	0	1	2	3	4

Response Surface Methodology (RSM)

Needles of *Pinus massoniana* were used to optimize the extraction conditions of PNEO by RSM. In the RSM experimental design, the extraction time, water dosage, and concentration of NaCl were selected as 3 independent variables, and the extraction yield of PNEO was taken as the response value. The Box-Behnken response surface method in Design-expert 11 software was used for experimental design and data processing.

Chemical Composition Analysis of PNEO

The extracted PNEO was diluted 20 times with ethanol, and the sample was filtered with a 0.22 μm organic filter head. The sample was transferred to the sample bottle for GC-MS (Bruker SCIONSQ-TQ, Karlsruhe, Germany) and GC (Nexis GC-2030, Kyoto, Japan) detection. The chemical components of PNEO were analyzed by the retrieval system in GC-MS, and the relative content of each chemical component was calculated by the area normalization method in GC. The GC-MS chromatographic column was a DB-5 capillary column (30 m × 0.32 mm × 0.25 μm). The detector was a hydrogen flame ionization detector (FID). The temperature of the column was held at 70 ℃ and maintained for 2 min, then increased at 3 ℃/min up to 160 ℃, and finally increased at 10 ℃/min up to 250 ℃ and maintained for 10 min. The split ratio was 1:50, and the carrier gas was nitrogen (99.999%). The temperature of the vaporization chamber and detector were 260 ℃ and 280 ℃, respectively. The injection volume was 0.50 μL. The electron bombardment source was EI, and the electron energy was 70 eV. The temperature of the ion source and transmission line were 230 ℃ and 270 ℃, respectively.

RESULTS AND DISCUSSION

Selection of Factors and their Levels by Single-Factor Analysis

Under the same extraction conditions, the extraction yields of samples broken by high-speed agitator and cut into 1-cm length with scissors were compared. The results showed that the extraction yield of the former was higher than that of the latter. The waxy protective film on the surface of pine needles was damaged after breaking, which was conducive to the release of essential oils. Therefore, broken samples were used in the subsequent tests.

Single factor experiments were used to analyze the effect of extraction conditions on the extraction yield of PNE, as shown in Fig. 1. Keeping the concentration of NaCl at 2% and the water dosage of 800 mL unchanged, the extraction yield increased gradually

with the extension of extraction time, but after the extraction time reached 2 h, the extraction yield began to decline slowly. When the extraction time was too short, the essential oil extraction was not sufficient. However, a very long extraction time resulted in the loss of volatile components and waste of resources. Therefore, the suitable extraction time was 2 h.

Keeping the concentration of NaCl (2%) and the extraction time (2 h) constant, and changing the water dosage, the extraction yield of PNEO increased first and then decreased. The extraction yield reached a maximum when the water dosage was 800 mL. When the amount of water dosage was too small, the distribution of pine needles in the system was uneven. As the water dosage increases, the system must absorb more heat to maintain the extraction process. Both of these conditions reduce the yield of PNEO.

Keeping the water dosage of 800 mL and the extraction time of 2 h unchanged, the effect of concentration of NaCl on the extraction yield was studied. The yield of PNEO was improved after using NaCl, but with the increase of the concentration of NaCl, the yield of PNEO increased first and then decreased. A certain concentration of sodium chloride solution is conducive to the extraction of PNEO because it promotes the release of essential oil into the solution and reduces the solubility of essential oil in water. However, when the concentration of NaCl was too high, other substances in cells were released into the solution, which hindered the extraction of PNEO. In addition, when the concentration of NaCl is too high, violent boiling of the solution may occur, resulting in the loss of volatile substances in PNEO (Dai *et al*. 2011).

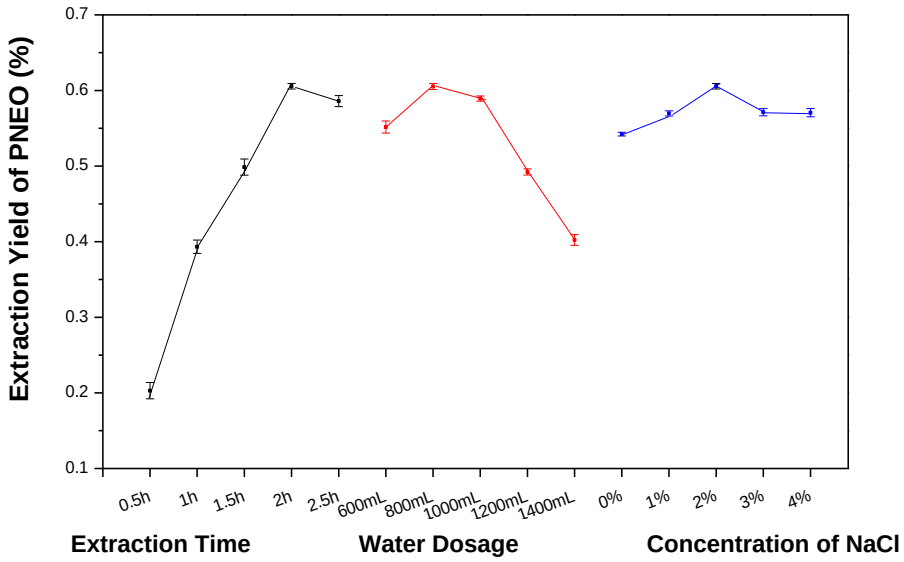

Fig. 1. Effect of extraction time, water dosage and concentration of NaCl on extraction yield of PNEOs

Model Fitting of Influencing Factors on Extraction Yield of PNEO

In the Box–Behnken design, the extraction time, water dosage, and concentration of NaCl were selected as independent variables, and the extraction yield of PNEO was the response value. The experimental design and results are shown in Table 2. The response value ranged from 0.463% to 0.609% according to each experiment design. The experiment conditions with the maximum extraction yield of PNEO were as follows: extraction time of 2 h, water dosage of 800 mL, and NaCl concentration of 2% (Run 16).

The quadratic model is the best fitting model. Statistical analysis of variance showed that this model had a large F-value (400.32) with a small P-value (< 0.0001), which implied the model is significant. Additionally, the *Lack of Fit* was not significant relative to the *Pure error* (P-value =0.2326＞0.05), which indicated that the quadratic model was valid, reliable, and accurate (Table 3) (Zhang *et al*. 2021).

Table 2. Experimental Design Matrix and Results

Run	A: Extraction time (h)	B: Water dosage (mL)	C: Concentration of NaCl (%)	Y: Yield (%)
1	2.0	600	1	0.522
2	2.0	800	2	0.605
3	2.0	1000	3	0.583
4	1.5	1000	2	0.463
5	1.5	600	2	0.446
6	2.5	600	2	0.521
7	2.5	800	1	0.553
8	2.0	800	2	0.601
9	2.5	800	3	0.572
10	2.5	1000	2	0.569
11	1.5	800	1	0.468
12	2.0	600	3	0.553
13	2.0	800	2	0.607
14	2.0	1000	1	0.568
15	2.0	800	2	0.607
16	2.0	800	2	0.609
17	1.5	800	3	0.476

Table 3. Statistical Analysis of Variance of the Quadratic Model

Source	Sum of Squares	df	Mean Square	F-value	P-value	
Model	0.0500	9	0.0056	400.32	< 0.0001	significant
A-extraction time	0.0164	1	0.0164	1181.49	< 0.0001	
B-water dosage	0.0025	1	0.0025	179.25	< 0.0001	
C-concentration of NaCl	0.0007	1	0.0007	48.05	0.0002	
AB	0.0002	1	0.0002	17.33	0.0042	
AC	0.0000	1	0.0000	2.18	0.1832	
BC	0.0001	1	0.0001	4.62	0.0688	
A^2	0.0222	1	0.0222	1602.91	< 0.0001	
B^2	0.0047	1	0.0047	338.79	< 0.0001	
C^2	0.0011	1	0.0011	76.78	< 0.0001	
Residual	0.0001	7	0.0000			
Lack of Fit	0.0001	3	0.0000	2.18	0.2326	not significant
Pure Error	0.0000	4	9.200E-06			
Cor total	0.0500	16				
Fit statistics for regression analysis						
Std. Dev.	Mean	R^2	Adjusted R^2	Predicted R^2	C.V. %	Adeq Precision
0.0037	0.5484	0.9981	0.9956	0.9796	0.6790	56.4382

According to a regression analysis of the experimental data, the extraction yield of PNEO could be expressed by the following equation,

$$Y=-1.31035+1.17990A+0.001309B+0.077725C+0.000077AB+0.0055AC-0.000020BC-0.290600A^2-8.35000E^{-7}B^2-0.015900C^2 \quad (2)$$

where Y is the extraction yield of PNEO, and A, B, C are the variables for extraction time, water dosage, and concentration of NaCl, respectively.

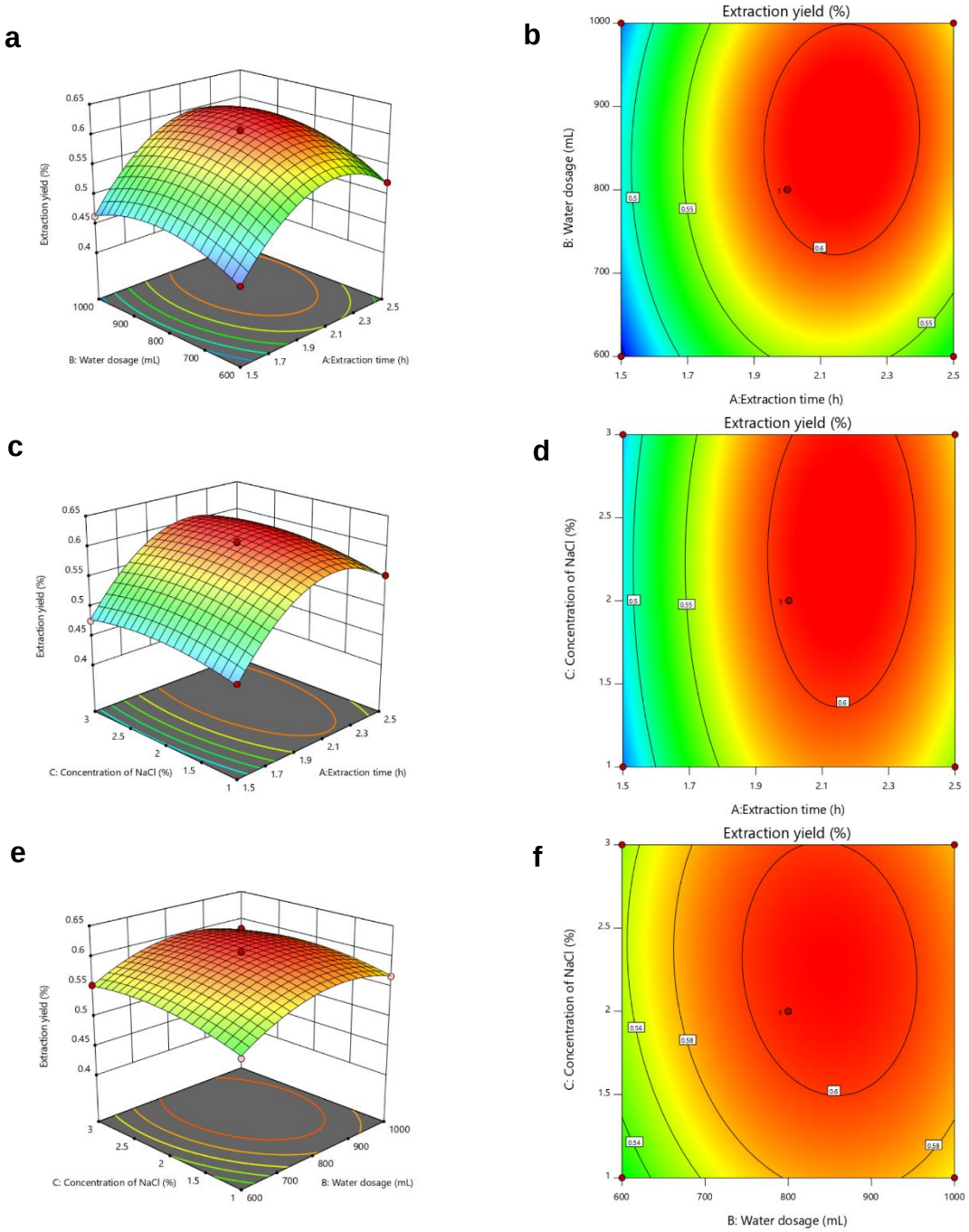

Fig. 2. 3D graphic surfaces and contour plots of the effects of extraction time, water dosage, and concentration of NaCl

The P-values less than 0.0500 indicated that the model terms are significant. In this case, *A*, *B*, *C*, *AB*, A^2, B^2, and C^2 were significant model terms. The value of R^2 (0.9981) was close to 1, which indicated that almost all of the variations found in the yield could be explained by the model. The *Predicted* R^2 value of 0.9796 was in reasonable agreement with the *Adjusted* R^2 of 0.9956, which suggested a strong correlation of the observed and anticipated data. The coefficient of variation (*C.V.%*=0.6790) indicated that the experimental data had a high degree of precision and sufficient reliability. *Adeq Precision* represented the signal to noise ratio, the value of this model was 56.438, indicated that the signal ratio was large enough (the ratio greater than 4 is desirable) (Rezzoug *et al*. 2005; Sodeifian *et al*. 2014; Elyemni *et al*. 2020; Ghadiri *et al*. 2020).

To display the synergistic effect of independent variables on the extraction yield of PNEO, three-dimensional response surface and two-dimensional contour plots were established, as shown in Fig. 2. The response surface and contour of the interaction between extraction time and water dosage on the extraction yield of PNEO are shown in Figs. 2a, b. The interaction between extraction time and water dosage had a significant effect on the extraction yield of PNEO. When the concentration of NaCl remained unchanged, the change rate of extraction yield with extraction time was greater than that with the water dosage, indicating that the extraction yield was mainly affected by extraction time. The extraction yield increased first and then decreased with increasing extraction time when the water dosage was constant as shown in Figs. 2c and 2d. The change rate of extraction yield with extraction time was greater than the change rate of concentration of NaCl. The extraction yield was mainly affected by the extraction time when combined with the concentration of NaCl. The interaction between water dosage and concentration of NaCl had no significant influence on the extraction yield as shown in Figs. 2e and 2f. The effect of water dosage on the extraction yield was greater than that of concentration of NaCl.

According to the design model, the theoretical optimum extraction conditions were as follows: an extraction time of 2.07 h, a water dosage of 854 mL, and a concentration of NaCl of 2.49%; the predicted value of extraction yield under this condition was 0.613%. Considering the operability of the extraction scheme in practical application, the modified conditions were as follows: an extraction time of 2 h, a water dosage of 850 mL, and a concentration of NaCl of 2.50%. The extraction yield of PNEO was 0.611% under the modified conditions, which was extremely close to the predicted value. There was a good correlation between experimental data and predicted value, which demonstrated that this model can accurately predict the yield of PNEO.

Difference of Extraction Yield of PNEO from Different Sources

The above experimental conditions optimized by RSM were used to extract the PNEOs of some common pines in Guangxi. The differences in the extraction yield of different pines are shown in Fig. 3. The extraction yield of A (*Pinus elliottii*), B (*Pinus elliottii*×*P. caribaea*), C (*Pinus latteri* Mason (from Vietnam)), D (*Pinus latteri* Mason (from China)), E (*Pinus yunnanensis* Franch. var. *tenuifolia* Cheng et Law), F (*Pinus yunnanensis*), G (*Pinus caribaea* Morelet var. *caribaea*), H (*Pinus caribaea* Morelet var. *bahamensis* Barrett et Golfari), I (*Pinus caribaea* Morelet var. *hondurensis* Barrett et Golfari), J (*Pinus massoniana*), K (*Pinus crassicorticea*), and L (*Pinus taeda*) were 0.413%, 0.434%, 0.466%, 0.478%, 0.404%, 0.235%, 0.335%, 0.336%, 0.321%, 0.611%, 0.604%, and 0.622%, respectively. The analysis of variance indicated that the yield of *Pinus taeda* was significantly higher than that of other pines except *Pinus massoniana*. The extraction yield of *Pinus yunnanensis* was significantly lower than that of its variant-*Pinus*

yunnanensis Franch. var. *tenuifolia* Cheng et Law. There was no significant difference in the extraction yield of *Pinus latteri* Mason originating from Vietnam and Hainan, China. For the three varieties of *Pinus caribaea*, there was no significant difference in their extraction yield. The composition and content of PNEO may be greatly affected by different geographical locations. All pine needle samples in this work were taken from the same experimental base, which ensured the consistency of site conditions. The results obtained by this way were more accurate and have comparative significance. *Pinus massoniana*, *Pinus crassicorticea*, and *Pinus taeda* had a higher content of PNEO than other pines. However, on the whole, the extraction yield of PNEOs were lower than that of *Cinnamomum cassia*, *Cinnamomum camphora*, *Litsea cubeba*, *etc*. (Chen *et al*. 2016). Therefore, the breeding of pines with a high content of PNEO needs to be investigated.

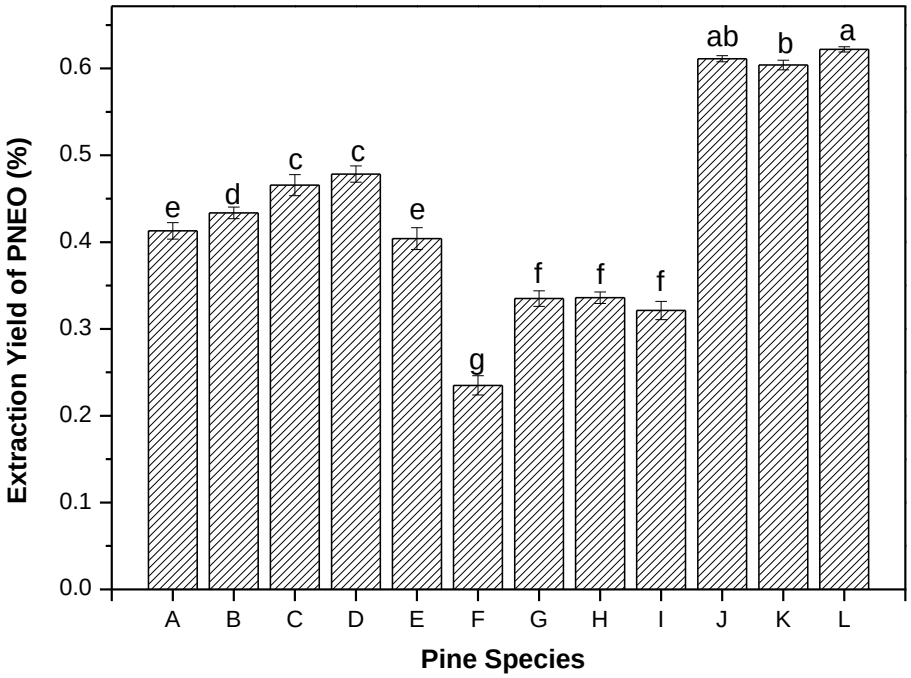

Fig. 3. Extraction yield of PNEO from different sources. (A, B, C, D, E, F, G, H, I, J, K, and L represent *Pinus elliottii, Pinus elliottii×P. caribaea, Pinus latteri* Mason (from Vietnam)*, Pinus latteri* Mason (from China)*, Pinus yunnanensis* Franch. var. *tenuifolia* Cheng et Law*, Pinus yunnanensis, Pinus caribaea* Morelet var. *caribaea, Pinus caribaea* Morelet var. *bahamensis* Barrett et Golfari*, Pinus caribaea* Morelet var. *hondurensis* Barrett et Golfari*, Pinus massoniana* Lamb., *Pinus crassicorticea* and *Pinus taeda* L., respectively. And the same below. The same letter indicates no significant difference at 0.05 level.)

Characteristics of Chemical Components of Different PNEOs

A total of 44 chemical compounds in 12 PNEOs were identified by GC-MS, and their relative contents are shown in Table 4. PNEOs were mainly composed of monoterpenes, sesquiterpenes, alcohols, and others (lipids, aldehydes and unidentified parts), among which monoterpenes and sesquiterpenes were the most important parts. The chemical components and relative contents of PNEOs from different sources were different. The common and relatively high content chemical components of 12 PNEOs were α-pinene, caryophyllene, germacrene, *etc*.

Table 4. Compositions and Relative Contents of PNEOs of Different Pines

No.	Chemical Compound	Relative Content (%)											
		A	B	C	D	E	F	G	H	I	J	K	L
1	Tricyclene	-	-	-	-	-	-	-	-	-	0.144	0.137	0.279
2	α-Pinene	4.976	0.437	3.455	8.917	17.524	14.068	1.312	1.931	3.390	38.332	29.168	43.442
3	Camphene	0.245	-	-	0.189	0.298	0.447	-	0.163	0.250	1.919	1.689	0.725
4	β-Pinene	20.114	3.247	0.508	1.760	3.670	20.932	0.311	6.074	7.098	10.446	10.600	18.799
5	Myrcene	0.545	-	1.445	1.006	0.444	1.165	1.164	1.394	2.122	1.110	0.996	1.240
6	α-Phellandrene	-	-	-	-	-	0.296	0.800	0.628	0.806	-	-	-
7	3-carene	-	-	6.011	7.321	-	-	-	-	-	-	-	-
8	Terpinolene	-	-	-	-	-	-	-	-	0.163	-	-	0.533
9	Limonene	-	-	-	-	-	-	-	-	-	5.574	4.960	6.692
10	β-Phellandrene	3.807	3.862	1.327	3.850	2.207	11.286	34.862	32.002	36.896	-	-	-
11	Ocimene	-	-	-	0.164	-	-	-	-	-	-	0.107	-
12	Terpinene	0.389	-	0.475	1.389	0.198	0.610	0.172	0.252	0.275	5.445	3.541	0.416
13	Linalool	-	-	0.143	-	-	-	-	-	0.195	0.125	0.337	0.162
14	4-Terpineol	-	-	0.203	0.141	-	0.206	0.252	0.138	0.200	0.118	-	0.162
15	α-Terpineol	1.015	0.364	-	0.139	0.257	0.550	0.248	0.500	0.519	0.557	0.293	0.739
16	Linalyl acetate	-	-	-	-	-	-	0.245	-	0.216	-	0.529	-
17	Bornyl acetate	0.579	0.335	-	-	-	0.279	2.648	0.924	1.084	0.294	0.443	0.265
18	δ-Elemene	0.181	-	-	-	-	-	0.487		0.232	0.274	0.245	0.257
19	Copaene	0.219	0.314	0.296	0.263	0.241	-	0.237	0.234	0.237	0.112	0.127	-
20	β-Bourbonene	-	-	-	-	0.280	0.237	-	0.203	0.172	0.112	0.185	-
21	β-Elemene	1.098	0.492	-	0.149	2.313	1.558	0.247	0.303	0.238	1.652	2.629	0.559
22	Lauric aldehyde	0.560	-	-	-	-	-	-	-	-	0.145	0.108	0.169
23	Caryophyllene	4.242	13.115	57.822	40.634	16.096	14.813	2.471	13.861	4.224	10.950	12.305	8.653
24	Humulene	0.864	2.406	10.436	6.459	2.794	2.590	0.497	2.464	0.818	1.996	2.345	1.828
25	β-Cadinene	-	-	0.347	0.140	0.221	-	-	-	-	0.231	0.142	-
26	Aromadendrene	0.199	0.278	0.174	0.144	0.261	-	0.286	0.188	0.172	0.108	0.153	-
27	Geranylene	30.483	54.180	6.658	22.042	16.075	8.738	22.370	24.804	25.849	2.018	1.865	2.775
28	α-Selinene	-	-	-	-	1.058	0.698	-	0.144	-	0.585	0.773	0.170
29	γ-Elemene	4.154	1.318	-	0.205	6.076	4.045	0.836	1.102	0.740	3.329	6.383	2.252
30	α-Muurolene	1.470	1.513	0.523	0.587	1.344	0.760	1.102	1.020	1.042	0.658	0.871	0.527
31	γ-Muurolene	1.884	1.714	0.241	0.309	2.569	1.234	1.476	1.167	1.178	1.099	1.535	0.676
32	δ-Cadinene	7.746	5.473	0.854	1.141	7.510	4.074	4.538	3.750	4.012	3.370	4.569	2.661
33	Spathulenol	0.279	-	-	-	1.402	0.637	-	-	-	0.591	0.967	0.388
34	Caryophyllene oxide	0.358	0.602	4.552	0.803	1.026	0.606	0.258	0.523	0.275	0.515	0.555	0.593
35	Globulol	0.325	-	-	-	0.164	0.226	-	0.166	-	0.214	0.167	1.817

36	Coubertol	0.283	-	-	-	0.319	-	0.272	0.143	0.162	0.188	0.252	-
37	t-Cubebol	4.292	2.198	0.335	0.368	4.428	2.221	2.673	1.687	1.767	2.363	3.264	-
38	α-Cadinol	0.713	0.642	0.161	0.150	0.715	0.370	0.677	0.419	0.446	0.352	0.490	0.299
39	α-Muurolol	6.137	2.983	0.477	0.517	5.881	3.190	3.345	2.164	2.038	3.141	4.104	2.306
40	Farnesol	-	-	0.133	-	0.115	0.476	-	-	-	-	-	-
41	Phenethyl benzoate	-	-	0.140	-	-	-	0.202	-	-	-	-	-
42	Manoyl oxide	-	-	-	-	-	-	0.377	-	0.182	0.115	-	-
43	Geranyl linalool	-	-	-	-	-	0.897	10.674	-	0.296	-	-	-
44	Viridiflorol	0.138	-	0.896	-	-	0.203	0.277	-	-	-	-	-
	Monoterpenes	30.076	7.546	13.221	24.596	24.341	48.804	38.621	42.444	51.000	62.970	51.198	72.126
	Sesquiterpenes	52.540	80.803	77.351	72.073	56.838	38.747	34.547	49.240	38.914	26.494	34.127	20.358
	Alcohols	13.182	6.187	2.348	1.315	13.281	8.976	18.418	5.217	5.623	7.649	9.874	5.873

The chemical composition and relative content of different PNEOs are summarized in Table 5. The PNEO of *Pinus elliottii* had the following characteristics: the relative content of sesquiterpenes (52.5%) was higher than that of monoterpenes (30.1%), and the content of alcohols was also higher (13.2%), β-pinene (20.1%) accounted for a high proportion of monoterpenes, and germacrene (30.5%) accounted for a high proportion in sesquiterpenes.

The PNEO of *Pinus elliottii*×*P. caribaea* had the following characteristics: the relative content of sesquiterpenes (80.8%) was higher than that of monoterpenes (7.5%), and germacrene (54.2%) accounted for a high proportion in sesquiterpenes. The PNEO of *Pinus yunnanensis* var. *tenuifolia* had the following characteristics: the relative content of sesquiterpenes (56.8%) was higher than that of monoterpenes (24.3%), and the content of alcohols was also higher (13.3%), α-pinene (17.5%) accounted for a high proportion of monoterpenes. The PNEO of *Pinus yunnanensis* had the following characteristics: the relative content of monoterpenes (48.8%) was higher than that of sesquiterpenes (38.7%), β-pinene (20.9%) accounted for a high proportion of monoterpenes.

The PNEOs of the three *Pinus caribaea* varieties were generally similar (β-phellandrene accounted for a high proportion of monoterpenes, germacrene accounted for a high proportion in sesquiterpenes). The biggest difference between them was that the content of alcohol substances (geranyl linalool) in PNEO of *Pinus caribaea* var. *caribaea* was much higher than that of *Pinus caribaea var. bahamensis* and *Pinus caribaea* var. *hondurensis*. The PNEOs of *Pinus latteri* from Vietnam and Hainan, China had the same characteristics as follows: the relative content of sesquiterpenes was higher than that of monoterpenes, caryophyllene accounted for a high proportion in sesquiterpenes. It is noteworthy that 3-carene components had been detected in PNEOs of *Pinus latteri*, which was not detected in PNEOs of other pines. The PNEOs of *Pinus massoniana*, *Pinus crassicorticea*, and *Pinus taeda* had similar characteristics (the relative content of monoterpenes was higher than that of sesquiterpenes and α-pinene accounted for a high proportion of monoterpenes). The main difference was that the relative content of isoterpinene in *Pinus taeda* was lower than that in *Pinus massoniana* and *Pinus crassicorticea*, and the relative content of γ-elemene in *Pinus crassicorticea* was higher than that in *Pinus massoniana* and *Pinus taeda*.

Combined with the extraction yield of PNEO, it is not difficult to find that the chemical compositions of PNEO with high extraction yield had the similar characteristics. The relative content of monoterpenes is higher than that of sesquiterpenes, while α-pinene accounts for a high proportion of monoterpenes. Solid phase microextraction-gas chromatography-mass spectrometry can accurately detect the content of volatile substances in needles. According to this characteristic, pine species with high content of PNEO can be quickly identified and selected without extraction. This finding provides a basis for selecting pine species with high content of PNEO based on the characteristics of chemical components.

The chemical components of PNEOs from different sources showed diversified characteristics, and their performance in the application needs to be further studied. In future research, the high-value utilization of the extracted needle residue should be considered.

Table 5. Characteristics of PNEOs of Different Pines

Pines	Characteristic									
	1	2	3	4	5	6	7	8	9	10
L	√			√						
J	√			√						
K	√			√						
I	√					√		√		
F	√				√					
H		√				√		√		
G		√				√		√	√	
D			√				√			√
C			√				√			√
E			√	√					√	
A			√		√			√	√	
B			√					√		

Notes: (Characteristic 1) the relative content of monoterpenes is higher than that of sesquiterpenes, (Characteristic 2) the relative content of monoterpenes is close to that of sesquiterpenes, (Characteristic 3) the relative content of monoterpenes is lower than that of sesquiterpenes, (Characteristic 4) α-pinene accounts for a high proportion of monoterpenes, (Characteristic 5) β-pinene accounts for a high proportion of monoterpenes, (Characteristic 6) β-phellandrene accounts for a high proportion of monoterpenes, (Characteristic 7) caryophyllene accounts for a high proportion in sesquiterpenes, (Characteristic 8) germacrene accounts for a high proportion in sesquiterpenes, (Characteristic 9) alcohols account for a relatively large proportion, (Characteristic 10) there is a unique component (3-carene) in PNEO.

CONCLUSIONS

1. Response surface methodology is an excellent and suitable method to optimize the extraction process of pine needle essential oils (PNEO). According to the Box-Behnken design model, the modified conditions were as follows: an extraction time of 2 h, a water dosage of 850 mL, and a concentration of NaCl of 2.50%. The extraction yield of PNEO was 0.611% under the modified conditions, which was extremely close to the predicted value.
2. The extraction yield of PNEO of different pines was different. The extraction yields of PNEO of *Pinus massoniana*, *Pinus crassicorticea*, and *Pinus taeda* were higher than other pines. The extraction yield of PNEO of *Pinus yunnanensis* was the lowest.
3. Monoterpenes, sesquiterpenes, and alcohols are the main components of PNEO. The chemical components of PNEO from different pines have their own features. The chemical components of PNEO with high extraction yield have the similar characteristic, pine species with high content of PNEO can be simply judged and selected according to this characteristic.

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REFERENCES CITED

Aikaterini, K., Samer, T., Rouba, S., Sofia, L., Olga, T., Vassilios, R., and Efstathia, I. (2021). "Antioxidant potential of pine needles: A systematic study on the essential oils and extracts of 46 species of the genus *Pinus*," *Foods* 10(1), 142. DOI: 10.3390/foods10010142

Anderson, G.W. (1985). "Green *Pinus radiata* needles as a feed for sheep," *Aust. J. Exp. Agric.* 25(3), 524-528. DOI: 10.1071/EA9850524

Belhachat, D., Mekimene, L., Belhachat, M., Ferradji, A., and Aid, F. (2018). "Application of response surface methodology to optimize the extraction of essential oil from ripe berries of *Pistacia lentiscus* using ultrasonic pretreatment," *J. Appl. Res. Med. Aromat. Plants* 9, 132-140. DOI: 10.1016/j.jarmap.2018.04.003

Chalchat, J., Garry, R., and Michet, A. (1985). "Sesquiterpenes of the essential oil of *Pinus sylvestris*," *Planta Med.* 51(3), 285-285. DOI: 10.1055/s-2007-969487

Chen, F., Du, X., Zu, Y., Yang, L., and Wang, F. (2016). "Microwave-assisted method for distillation and dual extraction in obtaining essential oil, proanthocyanidins and polysaccharides by one-pot process from *Cinnamomi cortex*," *Sep. Purif. Technol.* 164, 1-11. DOI: 10.1016/j.seppur.2016.03.018

Chen, Y., Liu, L., Wang, H., Ma, J., Peng, W., Li, X., Lai, Y., Zhang, B., and Zhang, D. (2021). "Environmentally friendly plant essential oil: Liquid gold for human health," *Adv. Agron.* 2021, 170, 289-337. DOI: 10.1016/bs.agron.2021.06.005

Dai, Y., Wu, W., Shi, H., and Huo, Q. (2011). "Study on extraction technology of essential oil from *Thuja occidentalis* L.," *Guangdong Agric. Sci.* 38(19), 101-103. DOI: 10.16768/j.issn.1004-874x.2011.19.007

Dob, T., Berramdane, T., and Chelghoum, C. (2005a). "Analysis of essential oil from the needles of *Pinus pinaster* growing in Algeria," *Chem. Nat. Compd.* 41(5), 545-548. DOI: 10.1007/s10600-005-0202-z

Dob, T., Berramdane, T., Dahmane, D., and Chelghoum, C. (2005b). "Chemical composition of the needles oil of *Pinus canariensis* from Algeria," *Chem. Nat. Compd.* 41(2), 165-167. DOI: 10.1007/s10600-005-0103-1

Elyemni, M., Louaste, B., Ouadrhiri, F. E., Bouia, A., and Eloutassi, N. (2020). "Application of response surface methodology to optimize the extraction of essential oil from *Rosmarinus officinalis* using microwave-assisted hydrodistillation," *J. Appl. Pharm. Sci.* 11(1), 129-136. DOI: 10.7324/JAPS.2021.110115

Feyza, O., Belma, A., Sahlan, O., and Senol, A. (2009). "Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia Ten.*," *Food Chem.* 112(4), 874-879. DOI: 10.1016/j.foodchem.2008.06.061

Gernandt, D. S., Geada, L. G., Ortiz, G. S., and Liston, A. (2005). "Phylogeny and classification of *Pinus*," *Taxon* 54, 29-42. DOI: 10.2307/25065300

Ghadiri, K., Raofie, F., Qomi, M., and Davoodi, A. (2020). "Response surface methodology for optimization of supercritical fluid extraction of orange peel essential oil," *Pharm. Biomed. Res.* 6(4), 303-312. DOI: 10.18502/pbr.v6i4.51

Hammami, S., Snène, A., Ridha, M. E. I., Faidi, K., Falconieri, D., Dhaouadi, H., Piras, A., Mighri, Z., and Porcedda, S. (2016). "Essential oil constituents and antioxidant activity of *Asplenium Ferns*," *J. Chromatogr. Sci.* 54(8), 1341-1345. DOI: 10.1093/chromsci/bmw071

Joseph, S. V. (2017). "Repellent effects of essential oils on adult *Bagrada hilaris* by using an olfactometer," *Southwest. Entomol.* 42(3), 719-724. DOI: 10.3958/059.042.0310

Moral, A., Aguado, R., Jarabo, R., and Tijero, A. (2017). "Cationized fibers from pine kraft pulp: Advantages of refining before functionalization," *Holzforschung* 71(11), 843-851. DOI: 10.1515/hf-2017-0023

Peach, D. A. H., Almond, M., Gries, R., and Gries, G. (2019). "Lemongrass and cinnamon bark: Plant essential oil blend as a spatial repellent for mosquitoes in a field setting," *J. Med. Entomol.* 56(5), 1346-1352. DOI: 10.1093/jme/tjz078

Rezzoug, S. A., Boutekedjiret, C., and Allaf, K. (2005). "Optimization of operating conditions of rosemary essential oil extraction by a fast controlled pressure drop process using response surface methodology," *J. Food Eng.* 71(1), 9-17. DOI: 10.1016/j.jfoodeng.2004.10.044

Sanja, C., Milka, M., Marija, E. S., Anesa, J. M., and Renata, B. (2008). "Chemical composition and antioxidant and antimicrobial activity of two *Satureja* essential oils," *Food Chem.* 111(3), 648-653. DOI: 10.1016/j.foodchem.2008.04.033

Shipra, J., Tamanna, T., Bharti, G., and Singha, A. S. (2021). "High-performance gum rosin-modified hyperbranched vinyl ester resin derived from multifunctional pentaerythritol," *Polym. Bull.* 79, 477-501. DOI: 10.1007/s00289-020-03511-x

Sodeifian, G., Azizi, J., and Ghoreishi, S.M. (2014). "Response surface optimization of *Smyrnium cordifolium boiss* (SCB) oil extraction *via* supercritical carbon dioxide," *J. Supercrit. Fluids* 95, 1-7. DOI: 10.1016/j.supflu.2014.07.023

Soltani, H. M., Sadat, N. S. A., and Vahid, S. J. (2018). "Essential oil profiling of Ajowan (*Trachyspermum ammi*) industrial medicinal plant," *Ind. Crops Prod.* 119, 255-259. DOI: 10.1016/j.indcrop.2018.04.022

Wang, X., Chen, X., Xie, X., Wu, Y., Zhao, L., Li, Y., and Wang, S. (2018). "Effects of thermal modification on the physical, chemical and micromechanical properties of Masson pine wood (*Pinus massoniana* Lamb.)," *Holzforschung* 72(12), 1063-1070. DOI: 10.1515/hf-2017-0205

Xie, J., Han, Q., Wang, J., Bai, L., Lu, J., and Liu, Z. (2019a) "Enhanced α-terpineol yield from α-pinene hydration via synergistic catalysis using carbonaceous solid acid catalysts," *Ind. Eng. Chem. Res.* 58, 22202-22211. DOI: 10.1021/acs.iecr.9b04848

Xie, J., Han, Q., Feng, B., and Liu, Z. (2019b). "Preparation of amphiphilic mesoporous carbon-based solid acid from Kraft lignin activated by phosphoric acid and its catalytic performance for hydration of α-pinene," *BioResources* 14(2), 4284-4303. DOI: 10.15376/biores.14.2.4284-4303

Zeb, U., Dong, W., Zhang, T., Wang, R., Shahzad, K., Ma, X., and Li, Z. (2019). "Comparative plastid genomics of *Pinus* species: Insights into sequence variations and phylogenetic relationships," *J. Syst. Evol.* 58, 118-132. DOI: 10.1002/jse.12492

Zeng, W., Zhang, Z., Cao, H., Jia, L., He, Q. (2012). "Chemical composition, antioxidant, and antimicrobial activities of essential oil from pine needle (*Cedrus deodara*)," *J. Food Sci.* 77(7), 824-829. DOI: 10.1111/j.1750-3841.2012.02767.x

Zermane, A., Larkeche, O., Meniai, A. H., Crampon, C., and Badens, E. (2014). "Optimization of essential oil supercritical extraction from *Algerian Myrtus communis L.* leaves using response surface methodology," *J. Supercrit. Fluids* 85, 89-94. DOI: 10.1016/j.supflu.2013.11.002

Zhang, H., Yan, H., Lu. C., Lin, H., and Li, Q. (2021). "Optimization of ultrasound and microwave-assisted extraction of sweet cherry tree branches and chemical component analysis by UPLC-MS/MS," *Trees* 35, 1247-1256. DOI:10.1007/s00468-021-02112-z

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