

Antioxidant Activity of Extracts from Simao Pine (*Pinus kesiya* var. *langbianensis*) Needles

Xurundong Kan ^{1,*}, Yaning Liu ², Li Qiu ³

¹ International Union Laboratory of China and Malaysia for Quality Monitoring and Evaluation of Agricultural Products in Yunnan, School of Biology and Chemistry, Pu'er University, Pu'er, China

² School of Tea (Pu'er), West Yunnan University of Applied Sciences, Pu'er, China

³ School of Architecture and Engineering, Yancheng Kindergarten Teachers college, Yancheng China

ABSTRACT

Simao pine is a dominant native tree species in Puer, Yunnan, China, with abundant underutilized pine needle resources. This study optimized the ultrasonic-assisted extraction of total flavonoids from Simao pine needles, and evaluated the in vitro antioxidant activity of the extracts. Single-factor experiments and orthogonal test showed that the optimal extraction conditions were solid-liquid ratio 1:20, extraction temperature 80°C, and extraction time 40 min, with a maximum total flavonoid yield of 6.86%. The total phenolic content of the extract was 10.97 µg per mg of dry extract. Antioxidant assays confirmed that the extract had a DPPH radical scavenging rate over 80% at 0.6–0.8 mg/mL, and a maximum hydroxyl radical ($\cdot\text{OH}$) scavenging rate of 72.29% at 0.8 mg/mL. The reducing power of the extract was significantly higher than that of vitamin C (Vc) and vitamin E (Ve) at 0.6–1.0 mg/mL. This study provides a theoretical basis for the development of Simao pine needles as a natural antioxidant resource.

KEYWORDS

Simao pine; Total flavonoids; Total phenols; Antioxidant activity; DPPH radical; Hydroxyl radical; Reducing power

1. INTRODUCTION

Pinus kesiya var. *langbianensis* (Simao pine) is a key economic forest species in Yunnan Province, with a planting area of over 20 million mu in Puer City alone [1]. At present, the utilization of Simao pine is mainly focused on wood and rosin production, while its pine needles, which are rich in bioactive components including flavonoids and polyphenols, are rarely developed and utilized, leading to a huge waste of natural plant resources.

Excessive accumulation of free radicals in organisms causes oxidative stress, which is closely linked to chronic diseases, aging and cellular damage [2]. Natural plant-derived antioxidants have gained extensive research attention due to their high safety, low toxicity and strong free radical scavenging capacity, compared with synthetic antioxidants [3]. Existing studies have verified the antioxidant potential of other pine needle extracts, but systematic research on Simao pine needle extracts remains insufficient [4-6]. This study optimized the flavonoid extraction process and tested the in vitro antioxidant activity of the extracts, aiming to provide reliable data support for the high-value utilization of Simao pine needle resources [7, 8].

2. MATERIALS AND METHODS

2.1. Materials and Reagents

Fresh Simao pine needles were collected from the campus of Puer University, dried at 80°C for 10 h, crushed and sieved through a 120-mesh sieve, then stored sealed at room temperature. Rutin, gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and other standard reagents were of analytical grade, purchased from regular commercial reagent companies.

2.2. Instruments

UV-visible spectrophotometer (Shimadzu UV-2600), electronic analytical balance (Mettler-Toledo ME204/02), constant temperature water bath, ultrasonic cleaner, rotary evaporator and other conventional experimental instruments.

2.3. Extraction and Determination Methods

Ultrasonic-assisted extraction was carried out with 75% ethanol as the extraction solvent. The extraction process was optimized by single-factor tests and $L_9(3^4)$ orthogonal test. Total flavonoid content was determined by aluminum nitrate-sodium nitrite colorimetry with rutin as the standard, and total phenolic content was determined by Folin-Ciocalteu method with gallic acid as the standard.

2.4. Antioxidant Activity Assays

Three classic in vitro antioxidant methods were adopted: DPPH radical scavenging assay, hydroxyl radical ($\cdot\text{OH}$) scavenging assay, and reducing power assay. The extract was prepared into gradient concentrations (0.2, 0.4, 0.6, 0.8, 1.0 mg/mL) for testing, and Vc and Ve were set as positive control groups in the reducing power test. All tests were repeated three times ($n=3$), and the experimental data were recorded and sorted.

3. RESULTS AND DISCUSSION

3.1. Standard Curves

The rutin standard curve for total flavonoids was $y=0.0112x-0.007$ ($R^2=0.999$), and the gallic acid standard curve for total phenols was $y=0.0715x+0.0092$ ($R^2=0.999$), both with good linearity within the test concentration range, ensuring the accuracy of subsequent content determination.

3.2. Optimization of Flavonoid Extraction Process

The orthogonal test was designed with three factors: solid-liquid ratio, extraction temperature and extraction time. The test factors, levels and results are shown in Table 1 and Table 2.

Table 1. Orthogonal experimental conditions

Level	A: Solid-liquid ratio	B: Extraction temperature (°C)	C: Extraction time (min)
1	1:15	60	20
2	1:20	70	30
3	1:25	80	40

Table 2. Flavonoid yield

Test No.	A	B	C	Total flavonoid yield (%)
1	1	1	1	4.49
2	1	2	2	4.73
3	1	3	3	5.56
4	2	1	3	6.27
5	2	2	2	6.54
6	2	3	1	6.07
7	3	1	2	4.53
8	3	2	1	3.99
9	3	3	3	4.64
k1	4.93	5.10	4.85	-
k2	6.29	5.09	5.27	-
k3	4.39	5.42	5.49	-
R	1.90	0.33	0.64	-

Range analysis showed that the influence order of factors on flavonoid yield was solid-liquid ratio > extraction time > extraction temperature. The optimal extraction condition was A₂B₃C₃ (solid-liquid ratio 1:20, temperature 80°C, time 40 min), and the verification test showed the total flavonoid yield reached 6.86%, proving the optimized process was reliable. The total phenolic content of the extract was 10.97 µg/mg dry extract, laying a material foundation for its antioxidant activity.

3.3. In Vitro Antioxidant Activity of Simao Pine Needle Extract

3.3.1. DPPH Radical Scavenging Activity

DPPH radical scavenging rate is a core index to evaluate the free radical scavenging ability of plant extracts. The test results of DPPH scavenging rate at different extract concentrations are listed in Table 3. The scavenging rate rose rapidly with the increase of extract concentration in the low concentration range, and tended to be stable after reaching 0.6 mg/mL, showing a strong free radical scavenging effect.

Table 3. The test results of DPPH scavenging rate

Extract concentration (mg/mL)	0.2	0.4	0.6	0.8	1.0
DPPH scavenging rate (%)	34.54	52.43	82.25	83.87	83.82

3.3.2. Hydroxyl Radical (•OH) Scavenging Activity

Hydroxyl radical is a highly active free radical that causes severe oxidative damage to organisms. The hydroxyl radical scavenging effect of the extract is shown in Table 4. The scavenging rate increased first and then decreased with the rise of concentration, reaching the peak value at 0.8 mg/mL, indicating the extract has a notable protective effect against hydroxyl radical-induced oxidative damage.

Table 4. The hydroxyl radical scavenging effect of the extract

Extract concentration (mg/mL)	0.2	0.4	0.6	0.8	1.0
•OH scavenging rate (%)	31.01	45.96	54.97	72.29	64.80

3.3.3. Reducing Power Comparison

Reducing power is positively correlated with antioxidant activity, and higher absorbance value represents stronger reducing capacity. The reducing power of the extract was compared with two common vitamin antioxidants (Vc and Ve), and the results are displayed in Table 5. The extract showed lower reducing power than Vc at low concentrations, but surpassed both Vc and Ve at 0.6 mg/mL and above, presenting a concentration-dependent enhancement trend.

Table 5. Comparison of Antioxidant Capacity

Concentration (mg/mL)	Simao pine extract (Absorbance)	Vc (Absorbance)	Ve (Absorbance)
0.2	0.4324	0.5241	0.4621
0.4	0.5521	0.5629	0.4847
0.6	0.6213	0.6032	0.5053
0.8	0.7428	0.6721	0.5831
1.0	0.8526	0.7621	0.6732

4. CONCLUSION

The optimal ultrasonic-assisted extraction conditions of total flavonoids from Simao pine needles are solid-liquid ratio 1:20, extraction temperature 80°C and extraction time 40 min, with a total flavonoid yield of 6.86% and total phenolic content of 10.97 µg/mg dry extract. The extract exhibits excellent in vitro antioxidant activity: DPPH radical scavenging rate exceeds 80% at 0.6-1.0 mg/mL, maximum hydroxyl radical scavenging rate reaches 72.29% at 0.8 mg/mL, and its reducing power is significantly higher than that of Vc and Ve at medium and high concentrations. These results confirm that Simao pine needles have great development potential as a natural antioxidant resource, and can be applied in food, cosmetics and related biomedical fields.

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