Study on Chemical Constituents and Antibacterial Activity of Volatile Oil from PrunusMume

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Abstract: The paper is to explore the best extraction process of volatile oil and extract the volatile oil from Prunusmume so as to analyze the constituents of the essential oil from Prunusmume. And the antibacterial activity of the volatile oil was tested. In the experiment, the volatile oil was extracted from the dried Prunusmume by steam distillation, and the extraction process was optimized by orthogonal experiment. The chemical composition of the volatile oil was separated and identified by gas chromatography-mass spectrometry (GC-MS). The antibacterial activity of volatile oil from Prunusmume was tested by pore and tube dilution method. And the best extraction process of volatile oil from Prunusmume was as follows: adding water for 20 times, soaking for 3 hours, extraction for 8 hours. Volatile constituents mainly contain Alkanes, aldehydes and esters. Volatile oil on the test with E.coli and Staphylococcus aureus has shown a good antibacterial effect. It can be concluded that the optimum extraction process of volatile oil from Prunusmume was stable and feasible. At the same time, it has a good antibacterial effect.

1. Introduction

Plum is the dried flower bud of the Rosaceae Prunus mume (Sieb.). It has a long history of application in China. And it has important value for seeing and appreciating medicinal properties [1]. Modern pharmacological studies have shown that Plum with Shugan Qi, tone stomach and the effect of phlegm Sanjie [2-3], plum flavonoids with free radicals inhibit the role of aldose reductase and anti-platelet aggregation [4-5]. Plum contains volatile oil, volatile oil with anti-inflammatory, anti-mutation, anti-cancer, insect repellent effect, enzyme inhibition, while spices, medical and health services and human health development has an important role [6-7]. Up to now, no research has been done on optimizing extraction conditions, main chemical components and antimicrobial activity of essential oil of Plum. The purpose of this experiment is to obtain the best extraction technology of the essential oil of Rhodiola rosea. It is also to separate and identify the main chemical components of the essential oil of Rhodiola rosea. And it’s to test the antibacterial activity of Rhodiola rosea oil. It is hoped that it can provide reference for the utilization of rich Rhizoma plum resources in nature.

2. Test materials and equipment

Red plum collected from the Jining Medical College. Associate Professor Wang Jianan identified as the Rosaceae plant Prunusmume (Sieb.). Red plum. Anhydrous ether, anhydrous sodium sulfate, ethyl acetate for the analysis of Staphylococcus aureus, Escherichia coli laboratory-preserved bacteria, nutrient agar, beef extract, beef peptone biological reagents, Agilent GC-MS5975 GC-MS (Agilent Technologies).

3. Test methods and results

Single factor test

3.1 The impact of adding water

Take mashed red plum about 50g, add the amount of distilled water followed by: 10, 15, 20, 25,

30 times, soaking 2h, extraction time was 6h. The purpose is to examine the different water volume of red plum essential oil extraction rate.

Table 1 Effect of water addition on extraction rate

Add water	10	15	20	25	30
Extraction rate	0.22	0.28	0.36	0.38	0.33

As can be seen from Table 1, the extraction rate of the essential oil of Rhodiola rosea is the highest under the condition of 25 times the amount of water added. Too little addition of water can make the plum infiltration insufficient. So the best condition is choosing 25 times for adding water.

3.2 Soaking time

Take mashed red plum about 50g, plus 25 times the distilled water, soaking time were: 0,1,3,5,7 h, extraction time was 6h, to examine the different soaking time plum volatile oil extraction rate.

Table 2 Effect of Soaking Time on Extraction Rate

Soaking time	0	1	3	5	7
Extraction rate	0.2	0.24	0.28	0.29	0.29

According to the results in Table 2, the extraction rate of volatile oil from Rhodiola rosea increased due to the lengthening of soaking time, reaching the highest value at 5h. According to soaking theory, soaking will make the plant cell gap becomes larger, and help to improve the oil yield of volatile oil.

3.3 The impact of extraction time

Take mashed red plum about 50g, plus 25 times the distilled water, soaked 3h, set the extraction time were: 4, 6, 8,10,12 h, compare the extraction time of the volatile oil extraction rate.

Table 3 Effect of Extraction Time on Extraction Rate

Extraction time	2	4	6	8	10
Extraction rate	0.24	0.28	0.31	0.32	0.32

As can be seen from the results in Table 3, the extraction rate of volatile oil in the distillation 8h larger, and then the distillation time tends to be stable, volatile oil extraction rate increased slowly, so we choosing 8h for the best distillation time.

4. Orthogonal test

4.1 Hongmei volatile oil extraction factor level design

Choose A water, B soaking time and C extraction time, the factors were selected three levels, respectively, the rate of volatile oil extraction is an indicator, the design of three factors and three levels of orthogonal test are shown in Table 4.

Table 4 Orthogonal factor level design

Level	Factor		
	A	B	C
1	20	3	4
2	25	5	6
3	30	8	8

4.2 Orthogonal experimental design

According to Table 5, each test were taken mashed Plum about 50g. The data were analyzed visually and analyzed by ANOVA using "Orthogonal Experiment Assistant" software [9]. The results are shown in Table 5 and Table 6.

Table 5 Orthogonal experimental design and results

Test number	Factor				Extraction rate (%)
	A	B	C	D	
1	1	1	1	1	0.32
2	1	2	2	2	0.36
3	1	3	3	3	0.36
4	2	1	2	3	0.32
5	2	2	3	1	0.36
6	2	3	1	2	0.20
7	3	1	3	2	0.32
8	3	2	1	3	0.24
9	3	3	2	1	0.28
K1	0.347	0.320	0.253	0.320	
K2	0.293	0.320	0.320	0.293	
K3	0.280	0.280	0.347	0.307	
R	0.067	0.040	0.094	0.027	

Table 6 Analysis of variance results

Source	SS	df	MS	F	Prominenc
AAdd water	0.007	2	1.120	4.60	Not
BSoaking time	0.003	2	0.480	4.60	Not obvious
CExtraction time	0.014	2	2.240	4.60	obvious
D R	0.03	8			

The visual analysis results in Table 5 show that RA = 0.067, RB = 0.040, RC = 0.094. C is the most important factor, which has the greatest impact on the test results, followed by the A and B factors. The order of the three factors is C> A> B. Analysis of variance shows that: extraction time C was significant. The C factor was statistically significant (P<0.05), while A and B had no significant difference (P> 0.05). Through Intuitive analysis of the results, we can think of the extraction time is the main factor affecting the extraction rate of red plum oil. An intuitive analysis in Table 5 shows that: kA1> kA2> kA3, kB1 = kB2> kB3, kC3> kC2> kC1. According to the preferred principle of volatile oil extraction process, that is to determine the optimal extraction process for essential oil of Rhodiola rosea A1B1C3. That is: adding water 20 times, soaking time 3h, extraction time 8h.

4.3 Red plum essential oil GC-MS analysis

Column temperature was programmed with a DB-1701 (30 mx 250 μm x 0.25 μm) capillary column (Agilent Technologies, Inc.). The column temperature was programmed at 60 °C for 3 min. After heating to 130° C at 10 °C/min, speed up to 190 ℃, maintained for 50min, then 10 ℃/min speed up to 250 ℃, hold 15min. Injection volume: 10 L; inlet temperature is 230 ℃, detector temperature is 250 ℃, carrier gas is nitrogen, constant flow rate 1mL/min, split ratio 20: 1.

Mass spectrometry conditions: ion source for the EI source, ionization energy 70eV, the ion source temperature is 230 ℃, scanning range 60 ~ 550 amu, solvent delay 3 min.

According to the above experimental conditions, the essential oil of Rhodiola rosea was analyzed by GC-MS, and the relative content of each component in the volatile oil was calculated by the normalization of peak area. 78 peaks were obtained and the NIST11 database was searched for component analysis and 52 of them were identified. Having identified the names of the ingredients,

the relative content shown in Table 7.

As can be seen from Table 7, the identified 53 compounds account for 99.5% of the total volatile components, mainly including a large amount of alkanes, aldehydes and esters. There are 5 more than 5%, n-eicosane, 2-methyl octadecane, n-pentacosane, benzaldehyde, benzyl benzoate.

5. Hongmei essential oil antibacterial test

Preparation of solid medium and liquid medium. E. coli and Staphylococcus aureus (SA) stored in the refrigerator were thawed and transferred to freshly prepared nutrient agar medium by plate scribing method, and cultured overnight in a 37 °C incubator. The two colonies on the medium were washed with 60ml.

5.1 Flat punch method

Using hole punching method, evenly spread on the nutrient agar plates with an applicator, and then evenly perforated with an agar punch, each plate 1 hole, we remove the agar in the hole. The red plum volatile oil drops into the hole to full. Overflow is appropriate, well labeled, overnight incubator at 37 ° C incubator, observe the inhibition zone size. Meanwhile, ethyl acetate was used as a negative control.

Table 8 Flat punch results

Sample solution	SAflat	E.coliflat
Hongmei volatile oil inhibition zone diameter / mm	15~18	14~18
Acetate inhibition zone diameter / mm	0	0

As can be seen from Table 8, the volatile oil of Rhodiola rosea has a significant inhibitory effect on Staphylococcus aureus and Escherichia coli, and the diameters of the bacteriostatic circles are respectively 15-18 mm and 14-18 mm. While the negative control ethyl acetate did not appear inhibition zone.

5.2 Test tube double gradient method

Table 9 Test tube double gradient dilution results

Test tube number	SALiquid turbidity	Test tube number	E.coliLiquid turbidity
1		11	
2		12	
3		13	
4		14	
5	+	15	
6	++	16	+
7	++	17	++
8	+++	18	+++
9	+++	19	+++
10	++++	20	++++
21	+++++	22	+++++
23	++++	24	++++

Twenty-four tubes of the well-handled test tubes were stuffed with test tube plugs and placed in a 37 °C. incubator overnight for culture. Observation and recording were performed to find out tube turbidity. The concentration of the test tube with test bacterial growth was taken as the minimum inhibitory concentration). The results are shown in Table 9.

According to the experimental results, No.5 test tube began to appear turbidity in tubes No.1 to

No.10, and its liquid volume concentration (v/v) was 0.03125. 11 to 20 tubes began to appear cloudy on the 16th test tube, the liquid volume concentration of 0.01563, empathy dehumidifier volatile oil minimum inhibitory concentration of Escherichia coli should be greater than this concentration. While the colonies in tubes 21, 22, 23, and 24 is negative.

6. Conclusions

In this paper, single factor experiments, respectively, the amount of water, soaking time and extraction time of three factors on the extraction rate of red oil were investigated. And through the three factors, each of the three levels are carried out the extraction process orthogonal optimization experiment. And then determine the amount of water 20 times, soaking 3h, extraction 8h optimal extraction parameters. The use of steam distillation extraction Rhododendron volatile oil is simple and convenient operation, with great feasibility. Red oil can play a guiding role in the production of volatile oil. The result of GC-MS showed that the volatile oil contained a large amount of alkanes, aldehydes and esters. The results of bacteriostasis showed that the volatile oil of Rhodiola rosea had obvious inhibitory effect on the two selected strains. In this paper, the antibacterial activity of the essential oil of Rhodiola rosea was tested simply and simply. Due to the complex diversity of essential oil components of Rhodiola rosea, the experiments on other biological activities and pharmacological effects are of great value and significance.

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Table 7 Chemical Constituents of Volatile Oil from PrunusMume lative content (%)

Serial number	Retention time (min)	Compound English name	CAS	Relative content (%)
1	6.2539	Benzaldehyde	000100-52-7	6.1318
2	7.5736	Benzyl alcohol	000100-51-6	0.0864
3	8.6966	1,6-Octadien-3-ol, 3,7-dimethyl-	000078-70-6	0.0653
4	8.764	Nonanal	000124-19-6	0.8358
5	9.6793	2-Nonenal, (E)-	018829-56-6	0.0498
6	9.7635	Acetic acid, phenylmethyl ester	000140-11-4	0.0936
7	11.246	Phenol, 4-(2-propenyl)-	000501-92-8	0.1128
8	11.6952	2-Propenal, 3-phenyl-	000104-55-2	0.0492
9	12.3859	Heptadecanal	1000376-70-0	0.1497
10	12.4252	2-Propen-1-ol, 3-phenyl-	000104-54-1	0.0609
11	12.6329	Phenol, 2,3,5,6-tetramethyl-	000527-35-5	0.0554
12	13.7841	Eugenol	000097-53-0	1.3827
13	14.1491	Benzene, cyclopropyl-	000873-49-4	0.0786
14	15.2328	Methyleugenol	000093-15-2	0.5309
15	15.7775	1,3,5-Cycloheptatriene, 2,3,4,5,7,7-hexamethyl-	074779-68-3	0.0689
16	16.8388	Acetic acid, cinnamyl ester	000103-54-8	0.1473
17	17.1757	5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	003796-70-1	0.1394
18	17.5239	Hexadecane, 2,6,10,14-tetramethyl-	000638-36-8	0.1109
19	18.894	trans-.beta.-Ionone	000079-77-6	0.0702
20	20.2473	Phenol, 2,4-bis(1,1-dimethylethyl)-	000096-76-4	0.1044
21	20.3484	Butylated Hydroxytoluene	000128-37-0	0.0566
22	24.1387	Dodecanoic acid	000143-07-7	0.1485
23	33.0614	Heptadecane	000629-78-7	0.1961
24	33.7858	Oleyl alcohol, methyl ether	1000352-68-0	0.0532
25	36.0656	Benzyl Benzoate	000120-51-4	5.5945
26	36.4138	Tetradecanoic acid	000544-63-8	0.0563
27	37.9074	Benzene, 1,1'-[1,2-ethanediylbis(oxy)]bis-	000104-66-5	0.1482
28	39.8672	2-Pentadecanone, 6,10,14-trimethyl-	000502-69-2	1.5617
29	40.7038	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	000084-69-5	0.3029
30	42.0684	Nonadecane	000629-92-5	1.2388
31	42.7029	trans-Geranylgeraniol	024034-73-9	0.2497
32	43.0679	Hexadecanoic acid, methyl ester	000112-39-0	0.1172
33	44.2696	Dibutyl phthalate	000084-74-2	0.2189
34	44.6907	Propanoic acid, 2-methyl-, 3-phenylpropyl ester	000103-58-2	2.7241
35	45.5049	n-Hexadecanoic acid	000057-10-3	0.2298
36	45.7239	Eicosane	000112-95-8	0.6036
37	46.4708	Octadecanal	000638-66-4	0.3475
38	48.4866	1,4-Butanedione, 1,4-diphenyl-	000495-71-6	1.5958

39	49.6771	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	000112-63-0	0.151
40	50.3846	Heneicosane	000629-94-7	29.3918
41	50.7496	Phytol	000150-86-7	0.7025
42	52.0861	9,12-Octadecadienoic acid (Z,Z)-	000060-33-3	0.0562
43	55.9101	Docosane	000629-97-0	1.2363
44	57.5329	Octadecanal	000638-66-4	0.4314
45	64.7261	Octadecane, 2-methyl-	001560-88-9	26.3333
46	75.7433	Tetracosane	000646-31-1	0.6076
47	76.26	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	000119-47-1	0.1088
48	93.016	Pentacosane	000629-99-2	9.2067
49				
50	100.3608	2-methyloctacosane	1000376-72-8	0.2627
51	103.073	Heptacosane	000593-49-7	4.3016
52	106.0042	Octadecane	000593-45-3	0.0954
53	109.5868	Octadecane, 2-methyl-	001560-88-9	0.8147