Study on Separation and Purification of Chemical Components of Dichloromethane from Pine Needle Extract

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Abstract. To study the chemical composition of the extract of cedar pine needle methylene chloride. Methods: The paper used silica gel and Sephadex LH-20 column chromatography to separate and purify, and carried out structural identification based on physical and chemical properties and spectral data. At the same time, gallic acid was preliminarily separated by high-performance liquid chromatography (HPLC), the separation conditions of gallic acid were determined, and the content change of gallic acid after tannin was acid-decomposed was determined, proving that the extract may contain decomposable hydrolysis Tannin or compound tannin. Results: Five compounds were isolated from the dichloromethane extract of cedar pine needles, which were identified as: ferulic acid (1), osthol (2), β-phenylacrylic acid (3), paeonol (4), Magnolol (5). Conclusion: All compounds except Compound 5 were isolated from this plant for the first time.

Keywords: Cedar pine needles, dichloromethane extract, chemical composition.

1. Introduction
Cedar is a general term for the cedar genus of pinaceae, also known as Himalayan cedar, Himalayan cedar, and cedar. There are four species of this genus, including C. deodara, C. libani, C. brevifolia and C. atlantica, which are intermittently distributed in the Himalayas, western Asia (Lebanon, Syria and Turkey), Cyprus and North Africa (Morocco and Algeria). Traditional Chinese medicine believes that cedar has the effect of removing wind and collaterals, reducing swelling and increasing muscles, and promoting blood circulation to stop bleeding. Modern pharmacology has confirmed that cedar has various pharmacological activities such as antispasmodic, analgesic, anti-inflammatory, anti-cancer, antibacterial, anti-viral and so on.

Cedar is very rich in resources in China. In order to effectively develop and use it, this paper is based on the lignan extracted from cedar heartwood and its compounds have obvious anti-cancer activity on a variety of cancer cells. The structural types, physicochemical properties and biological activities of the hormones were reviewed; the chemical composition of the cedar pine needle methylene chloride extract was compared with phytochemical techniques such as silica gel column chromatography, gel SephadexLH-20 column chromatography, and recrystallization Separation and purification of the system, and structural characterization based on the physical and chemical properties of the compounds, spectral data and related literatures. As a result, 19 compounds were isolated from the cedar pine needle
methylene chloride extract, and 5 of them were identified: ferulic acid (1), osthol (2), β-phenylacrylic acid (3), paeonol (4), magnolol (5) and other compounds [1].

2. Materials and methods

2.1. Instruments and materials
METTLER FP-62 Melting Point Analyzer; Varian INOVA-400 MHz Nuclear Magnetic Resonance Instrument; Brucker Daktronics APEX II Mass Spectrometer; R-200 Rotary Evaporator; BHZ-D(III) Circulating Water Vacuum Pump; ZF-20D Dark Box UV Analysis Instrument; BSZ-40 automatic partial collection instrument; Sephadex LH-20 dextran gel; silica gel GF254 for thin layer chromatography, silica gel for column chromatography. The reagents used are of analytical grade [2].

2.2. Extraction and Separation of Cedar Pine Needles
Dry cedar pine needles 6.5 kg, soaked with petroleum ether at room temperature for seven times (three days each time), and recover petroleum ether to obtain extract 145; continue to soak pine needles with dichloromethane at room temperature for seven times (three days each time), recovered two Chloromethane was extracted with 128 g, 160 g silica gel (80-140 mesh) was added to the extract for sample preparation, silica gel column chromatography, gradient elution system were: petroleum ether, petroleum ether-ethyl acetate (4:1), Petroleum ether-ethyl acetate (1:2), ethyl acetate-methanol (4:1), ethyl acetate-methanol (1:1), methanol, after repeated silica gel column chromatography, SephadexLH-20 column chromatography, Macroporous adsorption resin column chromatography and recrystallization to obtain compound 1-19. The flow chart is shown in Figure 1 below [3].

![Flow chart of cedar pine needle extraction](image_url)

**Figure 1.** Flow chart of cedar pine needle extraction
3. Chemical substance identification results

Compound 1: White flake crystals (petroleum ether-ethyl acetate), mp 138-139°C, easily soluble in chloroform, ethyl acetate, acetone, Libermann-BurChard reaction was positive. The TLC display of the three systems is a single point. Petroleum ether: ethyl acetate (7:3), Rf=0.40; petroleum ether: acetone (8:2), Rf=0.40; n-hexane: ethyl acetate (8:2), Rf=0.50; iodine vapor fumigation Brownish yellow, and the same as the standard TLC Rf value. 1H-NMR (CDCl3) δ: 0.7 1, 1.03 (3H, s) corresponding to the 18,19 angle methyl proton, 1.04 (3H, d, J = 9.42Hz, CH₃), 0.87 (3H, d, J = 4.5Hz, CH₃), 0.83 (3H, d, J=4.2Hz, CH₃), 0.85 (3H, d, J=4.3Hz, CH₃) correspond to the methyl proton signals at positions 21, 26, 27.29, 3.54 (1H , M, H-3), 5.37 (1H, d, J=5.08Hz, H-6) ethylenic signal indicates the presence of a propylene-type triple substituted double bond, 5.18 (1H, dd, J=9, 8.5Hz, H-22) 5.04 (1H, dd, J=9, 8.5Hz, H-23) ethylenic signal indicates the presence of a high propylene type 1,2-bismethine substituted double bond, 83.54 (1H, m, H-3) gives the oxymatrine signal, identified as ferulic acid, with the molecular formula C₁₀H₁₀O₄. as shown in picture 2.

![Figure 2. Molecular formula of ferulic acid](image)

Compound 2: Colourless powder (chloroform), easily soluble in acetone and chloroform, mp80–84°C. Under the ultraviolet lamp (254, 365nm), it showed blue-violet and bright white fluorescence respectively; the reaction of 10% sulfuric acid-ethanol was purplish red; the reaction of ferric chloride-potassium ferricyanide was negative. 1H-NMR (600MHz, CDCl₃) δ: 1.67 (3H, brs, 3'-CH₃), 1.84 (3H, brs, 3'-CH₃), 3.52 (2H, d, J=7.2Hz, H-1'), 3.92 (3H, brs, 7-OCH₃), 5.21~5.24 (1H, m, H-2'), 6.22 (1H, d, J=9.6Hz, H-3), 6.84 (d, 1H, J= 8.4Hz, H-6), 7.28 (d, 1H, J=8.4Hz, H-4), 7.61 (d, 1H, J=9.0Hz, H-5); 13C-NMR (150MHz, CDCl₃) δ: 161.3 (C-2, s), 160.1  (C-7, s), 152.7 (C-8a, s), 143.7 (C-4, d), 132.6 (C-3', s), 126.2 (C- 5, d), 121.1 (C-2', d), 117.9 (C-8, s), 112.9 (C-4a, s), 112.8 (C-3, d), 107.2 (C-6, d), 55.9 (7-OCH₃, q), 25.7 (3'-CH₃, q), 21.9 (C-1', t), 17.9 (C-5', q). Compound 3: colourless needle crystals (acetone), mp 111-113°C. Ferric chloride-potassium ferricyanide reaction was positive. 1H-NMR (400MHz, DMSO-d₆) δ: 10.60 (1H, brs, OH), 9.79 (1H, s, CHO), 7.76 (2H, d, J = 8.56Hz, H-2, 6), 6.93 ( 2H, d, J=8.56Hz, H-3, 5); 13C-NMR (100MHz, DMSO-d₆) δ: 190.9 (C=O), 163.3 (C-4), 132.1 (C-2, 6), 128.5 (C-1), 115.9 (C-3, 5). It has been identified as osthol and the chemical formula is C₁₅H₁₆O₃. As shown in Figure 3.

![Figure 3. The molecular formula of osthol](image)
Compound 3: colourless needle-like crystals (petroleum ether-ethyl acetate, easily soluble in chloroform, acetone, mp140-142°C. Libermann-burchard reaction is purple-red, suggesting that the compound is a ghost compound. No fluorescence under ultraviolet light, 10% Nong Yao SO; ethanol is purple-red, the three solvent system TLC expansion shows a single point. Chloroform: acetone (9.5:0.5), Rf0.6; petroleum ether: ethyl acetate (3:1), Rf=0.45; n-hexane: acetone (9:1), Rf=0.3. 1H-NMR spectrum in the high field region (δ0.56-2.12) can be seen saturated CH and CH: signal piled up into a mountain shape, at 8.3.22 (1H, brs) There is a proton signal on a carbon atom connected to oxygen at 8.5.21 (1H, brs). There is a proton signal attributable to an olefinic carbon atom. There are 29 carbon signals in the 13C-NMR spectrum, of which 8140.7 There are two olefinic carbon signals at (CH) and 121.7 (CH) respectively. It is identified as β-phenylacrylic acid and its molecular formula is C10H10O2. As shown in Figure 4.

![Figure 4. β-Benzene acrylic acid molecular formula](image_url)

Compound 4: White amorphous powder, easily soluble in chloroform and methanol [4]. Ferric trichloride-potassium ferricyanide appears blue, suggesting that the molecule contains a phenolic light group, mp 173-174°C. ESI-MS m/z: 420 [M]+, molecular weight is 420.1. In 1H-NMR spectrum, 84.74 (2H, d, J=4Hz), 84.29 (2H, dd, J=6.8, 9.2Hz), 83.91 (2H, due to the repetition of its signal and the hydrogen signal on OCH3, the coupling constant cannot be determined), 3.10 (2H, m), according to the 13C-NMR spectrum found that the corresponding carbon is 86.1, 71.8, 54.3, DEPT spectrum shows that These are tertiary carbon, secondary carbon and tertiary carbon, respectively, suggesting that the compound molecule may contain substituted bitetrahydrobarran ring structure fragments, and the substitution positions of the substituents are symmetrical. It is designated paeonol and its molecular formula is C9H10O3. As shown in Figure 5.

![Figure 5. Paeonol molecular formula](image_url)

Compound 5: colourless transparent bulk crystal (chloroform), mp 173 ~ 174°C. Easily soluble in chloroform and methanol. Ferric chloride-potassium ferricyanide is blue. EI-MS m/z: 418 [M]+. 1H-NMR (400 MHz, CDCl3) δ: 6.58 (4H, s, H-2, 6), 4.73 (2H, d, J = 3.5 Hz, H-7), 4. 28 (2H , Dd, J = 8.5, 6.5 Hz, H-9e), 3. 90 (2H, dd, J = 8.5, 3.5 Hz, H-9a), 3. 88 (12H, s, 4 × OCH3), 3.09 (2H, m, H-8); 13C-NMR (100 MHz, CDCl3) δ: 147.1 (C-3, 5), 134.2 (C-4), 132.0 (C-1), 102.6 (C-2, 6), 86.0 (C-7), 71.8
(C-9), 56.3 (4 × OCH₃), 54.3 (C-8). It is designated as magnolol and its molecular formula is C₂₉H₅₀O. As shown in Figure 6.

![Honokiol molecular formula](image)

**Figure 6.** Honokiol molecular formula

4. Separation results of gallic acid from pine needle high-efficiency liquid methylene chloride

4.1. Test method

The tannin content (in terms of mass fraction) in the extract was determined by F-D color development and ultraviolet spectrophotometry. Cedar tannin extraction: water extraction and alcohol extraction. Weigh the treated pine needles, add dichloromethane liquid-to-material ratio 14:1 (mL: g, the same below) and heat to reflux for 5 h in a high-pressure reaction kettle, filter under reduced pressure, concentrate by rotary evaporation to a thick paste, and vacuum dry. Test its tannin content. Determination of gallic acid in the extract before acid hydrolysis: Weigh 0.500 g of the dried extract, add high-efficiency liquid dichloromethane to dissolve and dilute to 50 mL, take 10 mL, and extract 3 times with an equal volume of ethyl acetate, Combine, dilute to 50 mL, filter with filter membrane as test solution 1.

4.2. Experimental results

The liquid-to-material ratio was 15:1 and heated to reflux for 3 h. The total extract yield and tannin mass fraction are shown in Table 1. It can be seen that the extract of the aqueous solution is superior to the ethanol solution in both the total extract yield and the tannin mass fraction. And the use of water as an extraction solvent reduces both the production cost and the risk in the production process [5].

| Type of solvent      | Total extract yield/% | Mass fraction of tannin in extract/% |
|----------------------|-----------------------|-------------------------------------|
| Distilled water      | 6.64                  | 15.07                               |
| Absolute ethanol     | 6.58                  | 14.79                               |

Table 1. Effect of extraction solvent on total extract yield and extract tannin mass fraction

It can be seen from Figure 7: under the same chromatographic conditions, the sample 1 before acid hydrolysis showed a small peak at 7 min; under the same chromatographic conditions, the sample 2 showed a peak at 7 min, 1 in the two figures Peak No. is the same as the peak-out time of Figure 7, which proves that this peak is the absorption peak of gallic acid. A new peak appears at 5 min in the figure, presumably due to an unknown substance that appears due to acid hydrolysis. From this, it can be proved that part of the tannin is decomposed into gallic acid under the action of H₂SO₄. It is speculated that the extract contains hydrolysed tannin or compound tannin which can be hydrolysed to gallic acid [6].
5. Conclusion
There are relatively few lignans isolated from cedar, which deserves further study. At present, only a few studies have reported on the chemical composition of the wood parts of the plant, but there are few studies on the chemical composition of the needles. In order to make better use of the rich cedar resources and enrich the natural product database in the cedar plant, this experiment The dichloromethane extraction part of the pine needle leaf has been systematically studied in order to fully elaborate its chemical composition, find an active structure, and lay a material foundation for further research and development of the plant.

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