

Studies on Chemical Constituents of *Radix Angelicae pubescentis*

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Abstract—Background: *Angelicae pubescentis* is the root of *Angelica pubescens Maxim. f. biserrata* Shan et Yuan. The main active ingredients are coumarin and volatile oil, and it has a variety of pharmacological activities such as antibacterial, antioxidant and anti-inflammatory. In this paper, the active components of the CO₂ supercritical extract of *Angelicae pubescentis* were studied. The results of this study will provide a scientific basis for the study of the chemical composition of *Angelicae pubescentis*. Purpose: To study the chemical constituents of supercritical extract of *Radix Angelicae pubescentis*. Method: The supercritical extract of *Radix Angelicae pubescentis* was separated by silica gel column chromatography to obtain monomers. The structures were identified based on physicochemical properties and spectroscopic data. Result: Eight compounds were separated from the supercritical extract of *Radix Angelicae pubescentis*, six of which were identified. They are Osthol, Columbianedin, Columbianetin, Columbianetin Acetate, Xanthotoxin and Bergapten. Conclusion: Xanthotoxin and Bergapten were isolated, which structure were similar. The standard substance of Osthol and Columbianedin were produced in order to offer the good material base for quality evaluation and pharmacodynamic study of *Radix Angelicae pubescentis*. Their purification were more than 99%. The results of this study are of great significance for exploring the effective material basis and medicinal value of the supercritical extract of *Radix Angelicae pubescentis*.

1. INTRODUCTION

There are many chemical components of *Radix Angelicae pubescentis*, domestic and foreign scholars have made a lot of research on it. So far, it has been found to contain coumarin, volatile oil, terpenes, sterols, organic acids, sugars and other components.

The main component of *Radix Angelicae pubescentis* is coumarin. So far, more than 70 kinds of coumarins have been isolated from *Ducuo*. They include simple coumarins, barking coumarins, picrancoumarins, dicoumarins, and some coumarin glycosides. The various coumarin structures are shown in Figure 1.

In addition, there are also differences in the main chemical components in different varieties of *Radix Angelicae pubescentis*. The root of *A. pubescens Maxim* contains Angelol, Angelicone, Glabralactone, Bergapten, Osthol^[1], Umbelliferone, Scoppoletin, Angelic acid, Tiglic acid, Palmitic acid, stearic acid, oleic acid, linolenic acid, phytanol, glucose and a small amount of volatile oil^[2]. The root of *H. lanatum Michx* contains Angelicin, Pimpinellin, Bergapten and other various barking coumarins. In addition to the above components, the leaves also contain psoralen and so on. Among them, the

main barking coumarins are bovine parsnips^[3]. It also contains 0.26-0.57% volatile oil. So far, the chemical components isolated from *Radix Angelicae pubescentis* have been far more than these. With the progress of the times and the gradual improvement of scientific and technological means, the chemical components in *Radix Angelicae pubescentis* will be studied more and more thoroughly.

In this experiment, we utilized column chromatography for the separation of coumarins on the basis of CO₂ supercritical extract. We prepared to product the relevant standard substance, The results of this study are of great significance for exploring the effective material basis and medicinal value of the supercritical extract of *Radix Angelicae pubescentis*.

2. MATERIALS AND METHODS

2.1 Materials

1) *Instruments and equipments:*

a) *XR4 micro melting point apparatus (Shanghai optical Instrument Factory) The thermometer is not corrected.*

b) *Burker-ARX-300 Nuclear magnetic resonance spectrometer and Burker-ARX-600 Nuclear magnetic*

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resonance spectromete (Analysis and Testing Center of Shenyang Pharmaceutical University)

c) Shimadzu UV-256-FW self-recording spectrophotometer (Central Laboratory of Liaoning University of Traditional Chinese Medicine)

d) LC-10A HPLC, SPD-10A Detector, LC-10A Pump (Department of Plant Chemistry of Liaoning University of Traditional Chinese Medicine)

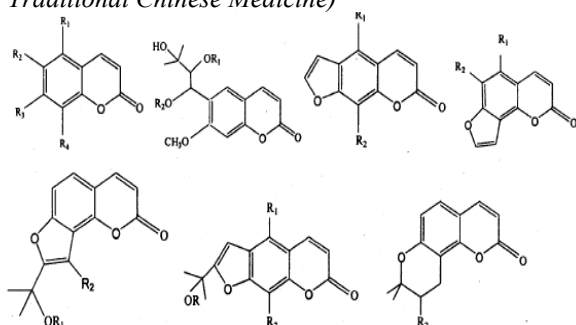


Figure 1. Structural classification of coumarin in Radix Angelicae pubescentis

2) Reagents: Silica gel (TLC, produced by Qingdao Marine Chemical Plant), Silica gel(Column, 100-200mesh, produced by Qingdao Marine Chemical Plant).

All chemicals for chromatography were of analytical grade. All chemicals for HPLC were of chromatographic grade.

2.2 Methods

In this experiment, we utilized column chromatography for the separation of coumarins on the basis of CO₂ supercritical extract.

1) Extract: We crushed the herbs of Radix Angelicae pubescentis. We take 1kg of crushed herbs between the 40-mesh screen and the 60-mesh screen, and then put them into the extraction tank. According to the CO₂ flow rate of 1.5 ml/min, extraction time of 20 minutes, pressure of 20 Mpa, temperature of 40°C, the operation was carried out to obtain the independent active supercritical extract.

2) Separation: Add 87g of supercritical extract of Radix Angelicae pubescentis into 100~200 mesh silica gel and stir them evenly. It was used for elution of mixture of petroleum ether and ethyl acetate. One stream fraction is collected every 500ml, and then solvent was recovered. After thin layer detection, flow fractions of similar composition were merged.

a) 25 to 30 flow fractions were placed stationary after they were combined. After solvent was dried, the mixture of petroleum ether and ethyl acetate was recrystallized to give a white needle-like crystal. It was defined as compound II.

b) 35 to 38 flow fractions were placed stationary after they were combined. After solvent was dried, the mixture of petroleum ether and ethyl acetate was recrystallized to give a white needle-like crystal. It was defined as compound I.

c) 46 to 48 flow fractions were placed stationary after they were combined. After solvent was dried, the mixture of petroleum ether and ethyl acetate was recrystallized to give a white needle-like crystal. It was defined as compound VI.

d) 52 to 54 flow fractions were placed stationary after they were combined. After solvent was dried, the mixture of petroleum ether and ethyl acetate was recrystallized to give a white needle-like crystal. It was defined as compound IV.

e) 60 to 62 flow fractions were placed stationary after they were combined. After solvent was dried, the mixture of petroleum ether and ethyl acetate was recrystallized to give a white needle-like crystal. It was defined as compound V.

f) 68 to 71 flow fractions were placed stationary after they were combined. After solvent was dried, the mixture of petroleum ether and acetone was recrystallized to give a white needle-like crystal. It was defined as compound III.

The process of separation is showed in Figure 2.

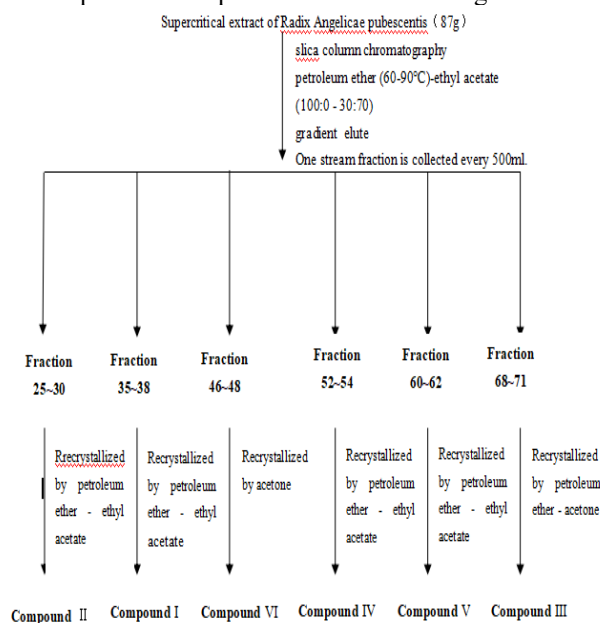


Figure 2. Structural classification of coumarin in Radix Angelicae pubescentis

3.RESULTS & DISCUSSION

In this experiment, we used CO₂ supercritical extraction technology to get the extract of monosacrum. We systematically separated the extracts and thus obtained eight compounds. We identified six of them by physicochemical properties and spectral data analysis. They includes five furan coumarins and one simple coumarins. They are Osthol, Columbianetin, Columbianetin Acetate, Xanthotoxin, Bergapten. The structures of the compounds were showed in Figure 3.

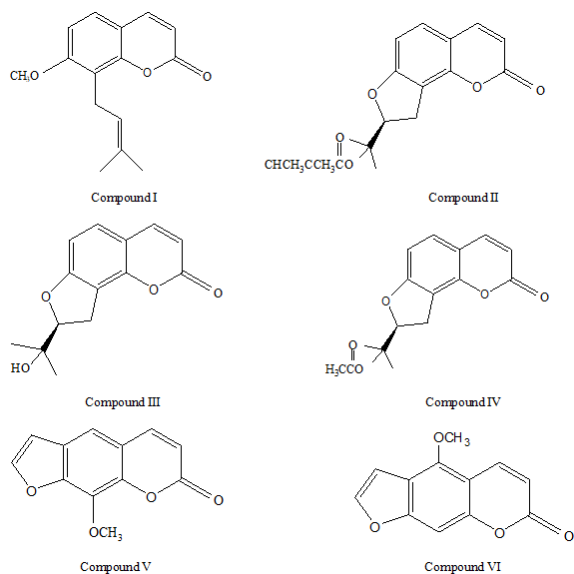


Figure 3. The structures of the compounds we separated in *Radix Angelicae pubescentis*

3.1 Chemical composition and structure identification of effective parts

1) *Compound I*: It is a white needle crystal (Petroleum ether - ethyl acetate), which m.p. is 82.2-82.5 °C. It showed blue fluorescence under ultraviolet light. It's physical constant and spectroscopic data is showed as follows.

a) $UV\lambda_{MeOH} \max_{nm}$: 257, 322.

b) $^1H_{NMR}$ ($CDCl_3$)(ppm): ξ 1.67 (3H, s), 1.84 (3H, s), 3.53 (2H, d, $J=7.3Hz$), 3.92 (3H, s), 5.22 (1H, t, $J=7.3Hz$), 6.23 (1H, d, $J=9.5Hz$), 6.84 (1H, d, $J=8.6Hz$), 7.29 (1H, d, $J=8.6Hz$), 7.60 (1H, d, $J=9.5Hz$).

c) $^{13}C_{NMR}$ ($CDCl_3$)(ppm): ξ 161.4, 160.1, 152.7, 143.7, 132.6, 126.2, 121.0, 117.8, 112.9, 107.3, 56.0, 25.7, 21.8, 17.9.

The physical constants and spectral data were consistent with those reported *Osthol* [4]. $^1H_{NMR}$ and $^{13}C_{NMR}$ spectral data of *Osthol* were showed in Table 1.

2) *Compound II*: It is a white needle crystal (Petroleum ether - ethyl acetate), which m.p. is 115-117 °C. It showed bluish violet fluorescence under ultraviolet light. It's physical constant and spectroscopic data is showed as follows.

a) $UV\lambda_{MeOH} \max_{nm}$: 326, 260, 265.

b) $^1H_{NMR}$ ($CDCl_3$)(ppm): ξ 1.60 (3H, s), 1.64 (3H, s), 1.67 (3H, s), 1.89 (3H, d, $J=1.5Hz$), 3.38 (2H, d, $J=8.5Hz$), 5.12 (1H, t, $J=8.5Hz$), 5.98 (1H, q, $J=1.5Hz$), 6.21 (1H, d, $J=9Hz$), 6.74 (1H, d, $J=8Hz$), 7.26 (1H, d, $J=8Hz$), 7.62 (1H, d, $J=9Hz$).

c) $^{13}C_{NMR}$ ($CDCl_3$)(ppm): ξ 167.0, 163.9, 161.0, 151.2, 144.0, 137.7, 128.8, 128.6, 113.5, 112.9, 112.1, 106.6, 89.2, 82.0, 27.6, 22.2, 21.2, 20.5, 15.6.

The physical constants and spectral data were consistent with those reported *Columbianedin* [5]. $^1H_{NMR}$ and $^{13}C_{NMR}$ spectral data of *Columbianedin* were showed in Table 2.

TABLE 1 $^1H_{NMR}$ AND $^{13}C_{NMR}$ SPECTRAL DATA OF OSTHOL ($CDCl_3$)

No. H-Proton	ξ (ppm)	No.C-Proton	ξ (ppm)
		2	161.4
H-3	6.23	3	112.9
H-4	7.60	4	143.7
H-5	7.29	5	126.2
H-6	6.84	6	107.3
		7	160.1
		8	112.9
		9	152.7
		10	117.8
H-1'	3.53	1'	21.8
H-2'	5.22	2'	121.0
		3'	132.6
H-4'	1.84	4'	25.7
H-5'	1.67	5'	17.9
OCH ₃	3.92	OCH ₃	56.0

TABLE 2 $^1H_{NMR}$ AND $^{13}C_{NMR}$ SPECTRAL DATA OF COLUMBIANEDIN ($CDCl_3$)

No. H-Proton	ξ (ppm)	No.C-Proton	ξ (ppm)
		2	163.9
H-3	6.21	3	112.1
H-4	7.62	4	144.0
H-5	7.26	5	128.6
H-6	6.74	6	106.6
		7	160.5
		8	113.5
		9	151.2
		10	112.9
H-1'	3.38	1'	21.8
H-2'	5.12	2'	88.7
		3'	85.3
H-4'	1.64	4'	20.5
H-5'	1.60	5'	21.2
		1''	167.0
		2''	128.8
H-3''	5.98	3''	138.6
H-4''	1.67	4''	15.6
H-5''	1.89	5''	27.6

3) *Compound III*: It is a colorless column crystal (Petroleum ether - ethyl acetate), which m.p. is 158.2-158.4 °C. It showed bluish violet fluorescence under ultraviolet light. It's physical constant and spectroscopic data is showed as follows.

a) $UV\lambda_{MeOH} \max_{nm}$: 328, 261.

b) $^1H_{NMR}$ ($CDCl_3$)(ppm): ξ 1.25 (3H, s), 1.37 (3H, s), 3.32 (2H, d, $J=8.3Hz$), 4.80 (1H, t, $J=8.3Hz$), 6.20 (1H, d, $J=9.5Hz$), 6.75 (1H, d, $J=8Hz$), 7.26 (1H, d, $J=8Hz$), 7.63 (1H, d, $J=9.5Hz$).

c) $^{13}C_{NMR}$ ($CDCl_3$)(ppm): ξ 163.6, 161.0, 151.2, 143.9, 128.7, 114.0, 113.0, 112.2, 106.6, 91.3, 71.7, 27.5, 25.9, 23.9.

The physical constants and spectral data were consistent with those reported *Columbianetin* [6]. $^1H_{NMR}$ and $^{13}C_{NMR}$ spectral data of *Columbianetin* were showed in Table 3.

4) *Compound IV*: It is a colorless prismatic crystal (Petroleum ether - ethyl acetate), which m.p. is 133.2-134.1 °C. It showed blue-purple fluorescence under ultraviolet light. It's physical constant and spectroscopic data is showed as follows.

a) $UV\lambda_{MeOH} \max_{nm}$: 250, 260, 325.

b) $^1\text{H NMR}$ (CDCl_3)(ppm): ξ 1.52 (3H, s), 1.58 (3H, s), 1.99 (3H, s), 3.28 (2H, m), 5.16 (1H, t), 6.21 (1H, d, $J=9.5\text{Hz}$), 6.75 (1H, d, $J=8\text{Hz}$), 7.28 (1H, d, $J=8\text{Hz}$), 7.65 (1H, d, $J=9.5\text{Hz}$).

c) $^{13}\text{C NMR}$ (CDCl_3)(ppm): ξ 170.1, 163.8, 160.9, 151.1, 143.9, 128.8, 113.3, 112.9, 112.1, 106.6, 88.6, 82.0, 27.5, 22.2, 21.8, 20.8.

The physical constants and spectral data were consistent with those reported Columbianetin Acetate^[7]. $^1\text{H NMR}$ and $^{13}\text{C NMR}$ spectral data of Columbianetin Acetate were showed in Table 4.

TABLE 3 $^1\text{H NMR}$ AND $^{13}\text{C NMR}$ SPECTRAL DATA OF COLUMBIANETIN (CDCl_3)

No. H-Proton	ξ (ppm)	No.C-Proton	ξ (ppm)
		2	163.6
H-3	6.20	3	112.2
H-4	7.63	4	143.9
H-5	7.26	5	128.7
H-6	6.75	6	106.6
		7	161.0
		8	114.3
		9	151.2
		10	113.7
H-1'	3.32	1'	23.9
H-2'	4.80	2'	91.9
		3'	73.3
H-4'	1.37	4'	27.5
H-5'	1.25	5'	25.9

TABLE 4 $^1\text{H NMR}$ AND $^{13}\text{C NMR}$ SPECTRAL DATA OF COLUMBIANETIN ACETATE (CDCl_3)

No. H-Proton	ξ (ppm)	No.C-Proton	ξ (ppm)
		2	163.8
H-3	6.21	3	112.1
H-4	7.65	4	143.9
H-5	7.28	5	128.8
H-6	6.75	6	106.6
		7	160.9
		8	113.3
		9	151.1
		10	112.9
H-1'	3.28	1'	22.2
H-2'	5.16	2'	88.6
		3'	82.0
H-4'	1.58	4'	27.5
H-5'	1.52	5'	20.8
		1''	170.1
H-2''	1.99	2''	21.8

5) Compound V: It is a white needle crystal (petroleum ether - ethyl acetate), which m.p. is 142-143.2 °C. It showed yellow fluorescence under ultraviolet light. Its physical constant and spectroscopic data is showed as follows.

a) $\text{UV}\lambda\text{MeOH maxnm}$: 299, 248.

b) $^1\text{H NMR}$ (CDCl_3)(ppm): ξ 4.27 (3H, s), 6.34 (1H, d, $J=9.5\text{Hz}$), 6.80 (1H, d, $J=2\text{Hz}$), 7.33 (1H, s), 7.67 (1H, d, $J=2\text{Hz}$), 7.75 (1H, d, $J=9.5\text{Hz}$).

c) $^{13}\text{C NMR}$ (CDCl_3)(ppm): ξ 160.4, 147.6, 146.6, 144.3, 142.9, 132.7, 126.1, 116.4, 114.6, 112.9, 106.7, 61.2.

The physical constants and spectral data were consistent with those reported Xanthotoxin^[7]. $^1\text{H NMR}$ and $^{13}\text{C NMR}$ spectral data of Xanthotoxin were showed in Table 5.

TABLE 5 $^1\text{H NMR}$ AND $^{13}\text{C NMR}$ SPECTRAL DATA OF XANTHOTOXIN (CDCl_3)

No. H-Proton	ξ (ppm)	No.C-Proton	ξ (ppm)
		2	160.4
H-3	6.34	3	112.1
H-4	7.75	4	144.3
H-5	7.33	5	112.9
		6	126.1
		7	146.6
		8	132.7
		9	142.9
		10	116.4
H-11	6.80	11	106.7
H-12	7.67	12	146.0
OCH_3	4.27	OCH_3	61.2

6) Compound VI: It is a white needle crystal (petroleum ether - ethyl acetate), which m.p. is 187-188 °C. It showed yellowish-green fluorescence under ultraviolet light. Its physical constant and spectroscopic data is showed as follows.

a) $\text{UV}\lambda\text{MeOH maxnm}$: 220, 247, 267, 308.

b) $^1\text{H NMR}$ (CDCl_3)(ppm): ξ 8.15 (1H, d, $J=9.7\text{Hz}$), 7.59 (1H, d, $J=2.3\text{Hz}$), 7.12 (1H, s), 7.02 (1H, d, $J=2.3\text{Hz}$), 6.27 (1H, d, $J=9.7\text{Hz}$), 4.27(3H,s).

c) $^{13}\text{C NMR}$ (CDCl_3)(ppm): ξ 161.3, 158.4, 152.7, 149.6, 144.8, 139.3, 112.7, 112.5, 106.4, 105.0, 93.8, 60.1.

The physical constants and spectral data were consistent with those reported Bergapten^[7]. $^1\text{H NMR}$ and $^{13}\text{C NMR}$ spectral data of Bergapten were showed in Table 6.

TABLE 6 $^1\text{H NMR}$ AND $^{13}\text{C NMR}$ SPECTRAL DATA OF BERGAPTEN (CDCl_3)

No. H-Proton	ξ (ppm)	No.C-Proton	ξ (ppm)
		2	161.3
H-3	8.15	3	112.7
H-4	6.27	4	139.3
		5	151.2
		6	112.5
		7	158.4
H-8	7.12	8	93.8
		9	149.6
		10	106.4
H-11	7.02	11	105.0
H-12	7.59	12	144.8
OCH_3	4.27	OCH_3	60.1

4. CONCLUSIONS

A systematic chemical separation of *Radix Angelicae pubescentis* CO₂ supercritical extracts was carried out, eight compounds were obtained, and their chemical composition was preliminarily studied. Six of them were identified by various chromatographic methods combined with spectral data. They are Osthol, Columbianedin, Columbianetin, Columbianetin Acetate, Xanthotoxin, Bergapten. Xanthotoxin and Bergapten were isolated, which structure were similar. The standard substance of Osthol and Columbianedin were produced in order to offer the good material base for quality evaluation and pharmacodynamic study of *Radix Angelicae pubescentis*. Their purification were more than 99%. The results of this study are of great significance for exploring the effective material basis and medicinal value of the supercritical extract of *Radix Angelicae pubescentis*.

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REFERENCES

1. Zhu Y, Li F, Liu Q, “Research progress of Chinese medicine alone living materia medica and pharmacology”, *Journal of Liaoning Economic Management Cadre College*, pp. 68-69, February 2010.
2. Qian C, Chen X, Xiao X et al. “High-throughput detection of the chemical composition of single active volatile oil based on full two-dimensional gas chromatography-quadrupole time-of-flight mass spectrometry,” *Journal of Analytical Testing*, vol. 41, pp. 78–90, January 2022.
3. Li J, Feng K, Wu S, et al. “Anti-inflammatory effect of columbianetin on lipopolysaccharide-stimulated human peripheral blood mononuclear cells,” *Mediators of Inflammation*, pp. 1-8. April 2018.
4. Guo X, Li D, Ma X, et al. “Determination of four chemical components in Duhuo decoction pieces,” *Modern Chinese Medicine*, vol 22, pp. 266-270, February 2020.
5. Cao J, Li C, “The textual research of herbal medicine and the pair of common medicines,” *Journal of Shaanxi University of Traditional Chinese Medicine*, vol 41, pp. 127-130, April 2018.
6. Zhou L, Zeng J, “Research progress on chemical composition and pharmacological activity of monoactive,” *Modern Chinese medicine*, pp. 1739-1748, December 2019.
7. GE A H, MA W F, WANG C P, et al. Ultra high performance liquid chromatography with photodiode array detector and quadrupole time-of-flight tandem mass spectrometry coupled with discriminant analysis to evaluate *Angelicae pubescentis radix* from different regions”. *J Sep Sci*, vol 37, pp. 2523-2534. May 2015.