Journal of Medicinal Plants Research Vol. 6(12), pp. 2520-2525, 30 March, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.513
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Isolation and purification of terpenoids from *Celastrus aculeatus Merr.* by high-speed counter-current chromatography

Yang Xie[1], Zongbao Ding[2], Wenjun Duan[2] and Qingsheng Ye[1]*

[1]School of Life Sciences, South China Normal University, Guangzhou 510631, People's Republic of China.
[2]School of Pharmaceutical Sciences, Southern Medical University, Guangzhou 510515, People's Republic of China.

Accepted 9 May, 2011

Following an initial clean-up step on a silica gel column, preparative high-speed counter-current chromatography (HSCCC) method was successfully established by using n-hexane–ethyl acetate–methanol–water (2.3:2:2:1.3, v/v) as the two phases solvent system to isolate and purify terpenoids from the stem and root bark of *Celastrus aculeatus Merr*. The isolation was done in less than 260 min and 2.5 mg of nimbidiol (I) and 3 mg of pristimerin (I I) were yielded from 250 mg of the crude extract with the purity of 95.0 and 97.1%, respectively, as determined by high-performance liquid chromatography (HPLC). Their structures were identified by using spectroscopic methods including ultraviolet (UV), electron ionization mass spectrometry (EI-MS), hydrogen nuclear magnetic resonance ^{1}(HNMR) and ^{13}CNMR. Nimbidiol and pristimerin were isolated from *C. aculeatus Merr* for the first time.

Key words: High-speed counter-current chromatography, terpeniods, nimbidiol, pristimerin, *Celastrus aculeatus Merr*.

INTRODUCTION

Celastrus aculeatus Merr.(*Celastrus*) (Guo et al., 2004; Kim et al., 1998; Jin et al., 2002; Westerheide et al., 2004) is a Chinese medicine that belongs to the family Celastraceae and the genus Celastrus. For centuries, roots, stems and leaves of *C. aculeatus* have been used to treat rheumatoid arthritis, osteoarthritis, lower back pain and so on. However, up to now, few phytochemical studies on this plant have been described in the literature. In order to find out the bioactive components, we have studied the chemical components of *C. aculeatus Merr*. Some of the bioactive components are difficult to separate because of their low contents. Although preparative HPLC or silica gel column makes the isolation and purification much easier than before, the considerable loss of herbal material requires more efficient methods to provide bioactive components.

*Corresponding author. E-mail: Ye-lab@scnu.edu.cn. Tel: 862085212021. Fax: 862085212021.

Compared to conventional liquid-solid methods, HSCCC without a solid phase has more advantages. A support-free liquid-liquid partition chromatographic technique eliminates such adsorption problems (Ito, 1981) has been widely used in preparative separation of natural products (Yang et al., 2009). In the present study, the HSCCC method was developed for the separation and purification of nimbidiol and pristimerin from *C. aculeatus Merr*. Their structures were elucidated with EI-MS, ^{1}HNMR and ^{13}CNMR. As far as we know, the two compounds were obtained from *C. aculeatus Merr* for the first time.

METHODOLOGY

Materials

The roots and stems of *C. aculeatus Merr*.(were collected from Guangdong province of China). TBE-300 A high speed counter-current chromatograph (Shanghai Tauto Biotech Company). HX-1050 constant-temperature circulation implements (Beijing Byoikang Lab. Instrument Company). Waters symmetry C_{18} column

Table 1. The partition coefficients (K) of the target compounds in different solvent systems with different ratios.

Hexane–ethylacetate–methanol–water			
(v/v)	K (Ⅰ)	K （Ⅱ）	K （Ⅲ）
0.4:2:0.4:2	0.63	0.12	0.82
1:2:1:2	6.97	4.65	2.33
2:2:2:2	7.58	12.64	2.89
2.3:2:2:1.3	1.32	0.45	1.56

(Waters Corp). Nuclear magnetic resonance spectrometer (600 M, Bruker Company). Fourier transforms infrared spectrometer (Nicolet Company). HPLC (Agilent Company). Milli-Q Academic ultra-pure water system (Millipore Company).

EXPERIMENTAL METHODS

Preparation of crude sample

The roots and stems of *C. aculeatus* Merr. were collected in the Guangdong province of China and identified by Dr. Ye Hua-gu, a plant taxonomist at South China Institute of Botany. A voucher specimen number 10943 was deposited there. Six kilograms air-dried roots and stems were minced with a grinder and then extracted three times with 95% ethanol at the temperature of 70 Celsius degrees every 3 h. After evaporating of the solvent under reduced pressure, the ethanol extract was diluted in H_2O and then extracted with petroleum ether. The petroleum ether layer (65 g) was chromatographed on a silica gel column using petroleum–ethyl acetate (10:1; 5:1; 1:1; 1:5) as eluent to give four fractions. Fraction 4 (12 g) was used for the subsequent HSCCC isolation and purification.

Measurement of partition coefficient (K)

The composition of the two phase's solvent system was selected according to the partition coefficient (K) and peak resolution of the target compounds. The measurement of K values was performed as following procedures according to the literature (Ito, 2005): 3 mg crude sample was weighed into a test tube to which 1mL of each phase of the pre-equilibrated two phases solvent system was added. The test tube was then shaken vigorously for 3 min to thoroughly equilibrate the sample between the two-phase. An aliquot of each phase (500 µL) was delivered into a test tube separately and evaporated to dryness. The residues were diluted with methanol to1mL and analyzed by HPLC. The K value was defined as the peak area of target compound in the upper phase (stationary phase) divided by the peak area of compound in the lower phase (mobile phase).

Preparation of two phase's solvent system

In the present study, the two phases solvent system composed of hexane/ethyl acetate/methanol/water (2.3:2:2:1.3, v/v) was used for HSCCC separation. The solvent mixture was shaked thoroughly at room temperature in a separatory funnel and placing overnight quietly. Before using, the separated two phases were degassed for 30 min, respectively.

HSCCC separation procedure

In the separation process the HSCCC column was first entirely filled with the upper organic phase. Then the aqueous mobile phase was pumped through the column at a flow rate of 1.5 mL/min in the head to tail direction (reversed mode). After the system reached hydrodynamic equilibrium, the crude sample dissolved in upper phase was injected through the sample port. The column was rotated at 900 rpm. The effluent from the outlet of the column was monitored continuously with the UV detector at 254 nm and collected in test tube per 5 min. Each peak fraction was collected and evaporated under reduced pressure. The residues were dissolved in methanol for subsequent HPLC analysis. After separation, retention of the stationary phase was measured by collecting the column contents into a graduated cylinder by forcing them out of the column with pressurized nitrogen gas.

HPLC analysis and identification of HSCCC peak fractions

The crude extract and fractions separated by HSCCC were analyzed by HPLC. HPLC was performed using a waters system with a waters symmetry C_{18} column (150×4.6 mm and 5.0 µm). The wavelength for detection was set at 254 nm according to the UV spectrums of all the fractions. Flow rate was 0.8 mL/min. Compound(I) was run in 30 min with methanol from 70 to 100% (v/v) and water 30% to 0 (v/v) in linear change. Compound (I I) was run in 30 min with methanol: 0.1%phosphoric acid (88:12) as mobile phase, and the column temperature was maintained at 30°C. The purities of the collected fractions were determined by HPLC. Identification of the HSCCC peak fractions was performed by ^{1}H NMR and^{13}C NMR.

RESULTS AND DISCUSSION

Selection of two phases solvent system and other conditions of HSCCC

The search for the suitable solvent system which gives a proper range of K values (partition coefficient) for the target compound is the crucial first step for successful HSCCC separation (Ito, 2003). The ideal K values of target compounds should be from 0.5 to 2.0. Generally, small K values result in poor peak resolution, while large K values tend to cause excessive band broadening. For this crude sample, the most commonly used Hexane–ethylacetate–methanol–water (HEMW) system at various volume ratios (0.4:2:0.4:2; 1:2:1:2; 2:2:2:2; 2.3:2:2:1.3, v/v) were further tested and their K values were measured. The K values of compounds in different ratios of HEMW determined by HPLC were listed in Table 1. As shown in Table 1, when HEMW (0.4:2:0.4:2, v/v) was

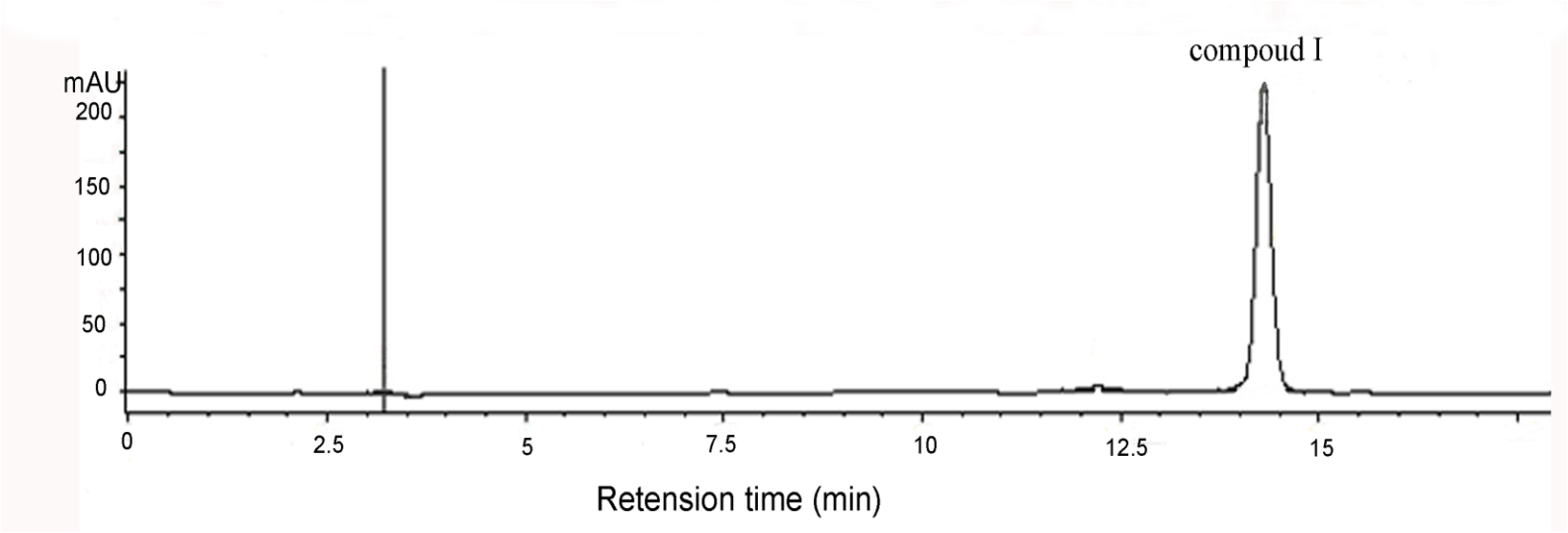

Figure 1. The HPLC chromatogram of compound I. Column: Waters symmetry C_{18} column (150×4.6 mm); column temperature: 25°C: mobile phase: methanol: water from 70:30 to100:0, flow rate: 0.8 mL /min; detection wave length: 254 nm.

selected as a solvent system, too small K values would result in poor peak resolution. It can be also seen that the K values of the HEMW (1:2:1:2, v/v) and HEMW (2:2:2:2, v/v) were too large, which lead to a long separation time and broad peaks. HEMW (2.3:2:2:1.3, v/v) was finally chosen for HSCCC separation as it gave a reasonable range of K values and a better resolution of target compounds. And it was used to isolate and purify two compounds from *C. aculeatus Merr* shown in Figure 3.

In addition, other factors such as the revolution speed of the separation column and the flow rate of the mobile phase were also investigated. The result indicated that a low flow rate could produce a good separation, but long elution time was required and peaks became broader. Experiments result showed that when the flow rate was set at 2.0 ml min^{-1}, revolution speed was 900 rpm, retention percentage of the stationary phase could reach 67% and good separation results could be achieved. Two compounds (I and II) were obtained in one-step elution less than 260 min (HSCCC chromatogram is shown in Figure 3) with the solvent system composed of n-hexane–ethylacetate–methanol-water (2.3:2:2:1.3, v/v). An amount of 2.5 mg of compound I and 13.0 mg of compound II were separated from 250 mg of the crude extract.

HPLC analysis and structure identification of HSCCC peak fractions

As shown in Figure 1 and 2, the HPLC chromatogram of each HSCCC peak fraction revealed that two compounds were purified from the crude extract. The purities of compound I and compound II were 95.0 and 97.1%, respectively. The structure identification of the HSCCC peak fractions was based on EI-MS, ^{1}H NMR and ^{13}C NMR. Data of compound I:

Molecular formula $C_{17}H_{22}O_3$. mp 171 to 175°C. HR-MS (EI) m/z: 274.1524 m/z (rel. int.%): 274 (M^+, 99), 259 (100), 217 (32), 203 (24), 191 (78), 189 (94), 177(57), 163(23), 69 (51), 57(30), 55(24);^{1}H-NMR ($CDCl_3$, 600 MHz) δ:7.62 (1H, br.H-14), 6.83 (1H, br.H-11), 2.54-2.66 (2H,dd,H-6), 2.15(1H, d,J = 12.6Hz,H-5), 2.15(1H, m), 1.71(H-a,d,J = 13.8Hz, H-1) ,1.64(H-b,m，H-1), 1.48(H-a,d,J = 13.2Hz，H-2), 1.78(H-b,dd,J = 13.2Hz, 4.2 Hz, H-2), 1.22 (H-a,m,H-3), 1.43 (H-b,m,H-3),1.14(3H, s, H-17), 0.94(3H, s, H-16), 0.88(3H, s, H-15)。^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 200.6 (C-7), 152.4 (C-13), 151.4 (C-12), 142.2(C-9), 123.7 (C-8), 113.6 (C-14), 110.1 (C-11), 49.8

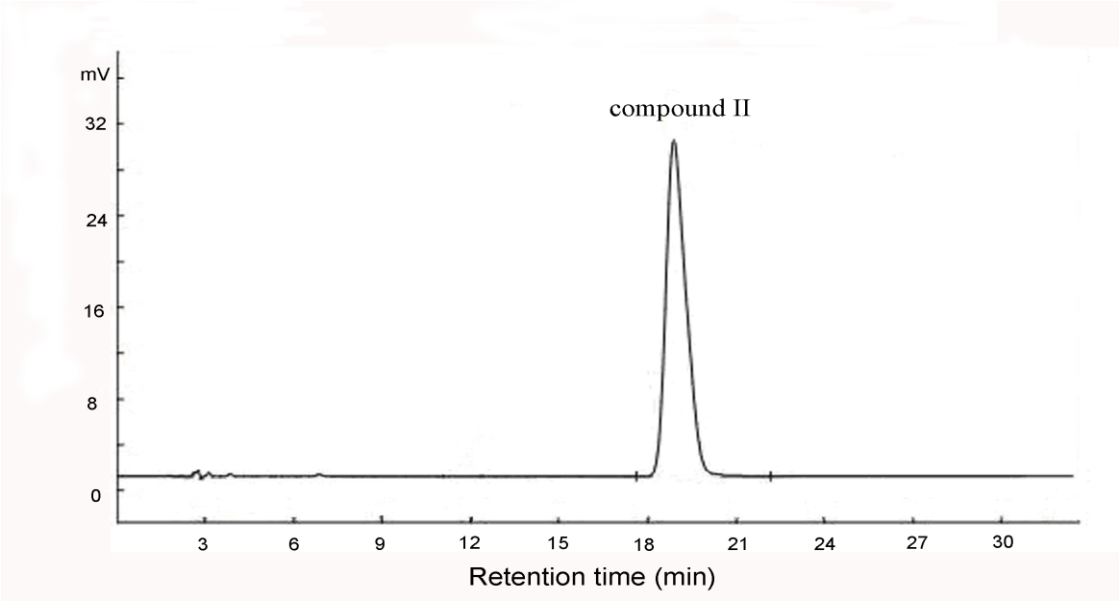

Figure 2. The HPLC chromatogram of compound II. Column: Waters symmetry C_{18} column (150×4.6 mm); column temperature: 25°C; mobile phase: methanol: 0.1%phosphoric acid (88:12), flow rate: 0.8 ml/ min; detection wavelength: 254 nm.

(C-5), 41.3(C-3), 37.9 (C-1), 37.8 (C-10), 35.9 (C-6),33.2 (C-4), 32.5 (C-2), 23.2 (C-18), 21.3 (C-19), 18.9 (C-20)。IR (KBr, cm^{-1}): 3428, 2990,2915, 2820, 1645, 1590, 1505, 1430, 1355, 1323, 1278, 1188, 875. This spectral data of ^{1}H NMR and ^{13}C NMR are in agreement with that of nimbidiol in the literature (Majumder et al., 1987; Masahiro et al., 2010).

Data of compound I molecular formula $C_{30}H_{40}O_4$. mp 217 to 219°C. EI- MS m/z 487$[M+Na]^+$, 465$[M+H]^+$。IR ($KBru_{max}$ / cm^{-1}): 3350, 2940, 1723, 1650, 1590, 1520, 1435, 1375,1300,1245,1220, 1205, 1185, 155, 1145, 1095, 1085, 990, 870, 860, 850。^{1}H-NMR ($CDCl_3$): δ 7.03 (1H, d, J=9.2Hz, H-6), 6.98 (1H, s, H-1), 6.36 (1H, d, J=7.2Hz, H-7), 6.53 (1H, brs, disappearing after the exchange of D_2O, -OH), 3.55 (3H, s, H-31), 2.22 (3H, s, H-23), 1.45 (3H, s, H-25),1.26 (3H, s, H-26), 1.18 (3H, s, H-30), 1.08 (3H, s, H-28), 0.52 (3H, s, H-27)。^{13}C-NMR：δ 119.8 (C-1), 178.6 (C-2), 146.2 (C-3), 117.3 (C-4), 127.6 (C-5), 133. 2(C-6),118.3 (C-7) 160.3 (C-8), 43.2 (C-9) 164.9 (C-10) 33.8 C-11) 29.9 (C-12) 39.6 (C-13), 45.3 (C-14), 28.9 (C-15), 36.6 (C-16), 30.8 (C-17), 44.5(C-18), 31.1 (C-19)，40.6 (C-20), 30.1 (C-21), 35.0 (C-22), 10.5 (C-23), 38.5 (C-25), 21. 8(C-26), 18.5 (C-27), 31.8(C-28), 178.9 (C-29), 32.9 (C-30), 51.8 (C-31). This spectral data of ^{1}H NMR and ^{13}C NMR are in agreement with that of pristimerin in the literature (Jiang et al., 1996).

CONCLUSION

HSCCC was successfully used for the preparative isolation of terpenoids. In combination with a suitable extraction and cleanup procedure prior to HSCCC separation, 2.5 mg nimbidiol and 3.0 mg pristimerin were obtained from 250 mg of the crude sample on a preparative scale. Meanwhile, the isolation of nimbidiol and pristimerin for the first time from *C. aculeatus Merr* proved HSCCC to be a useful tool for the exploring of the chemical component of traditional medicinal herbs.

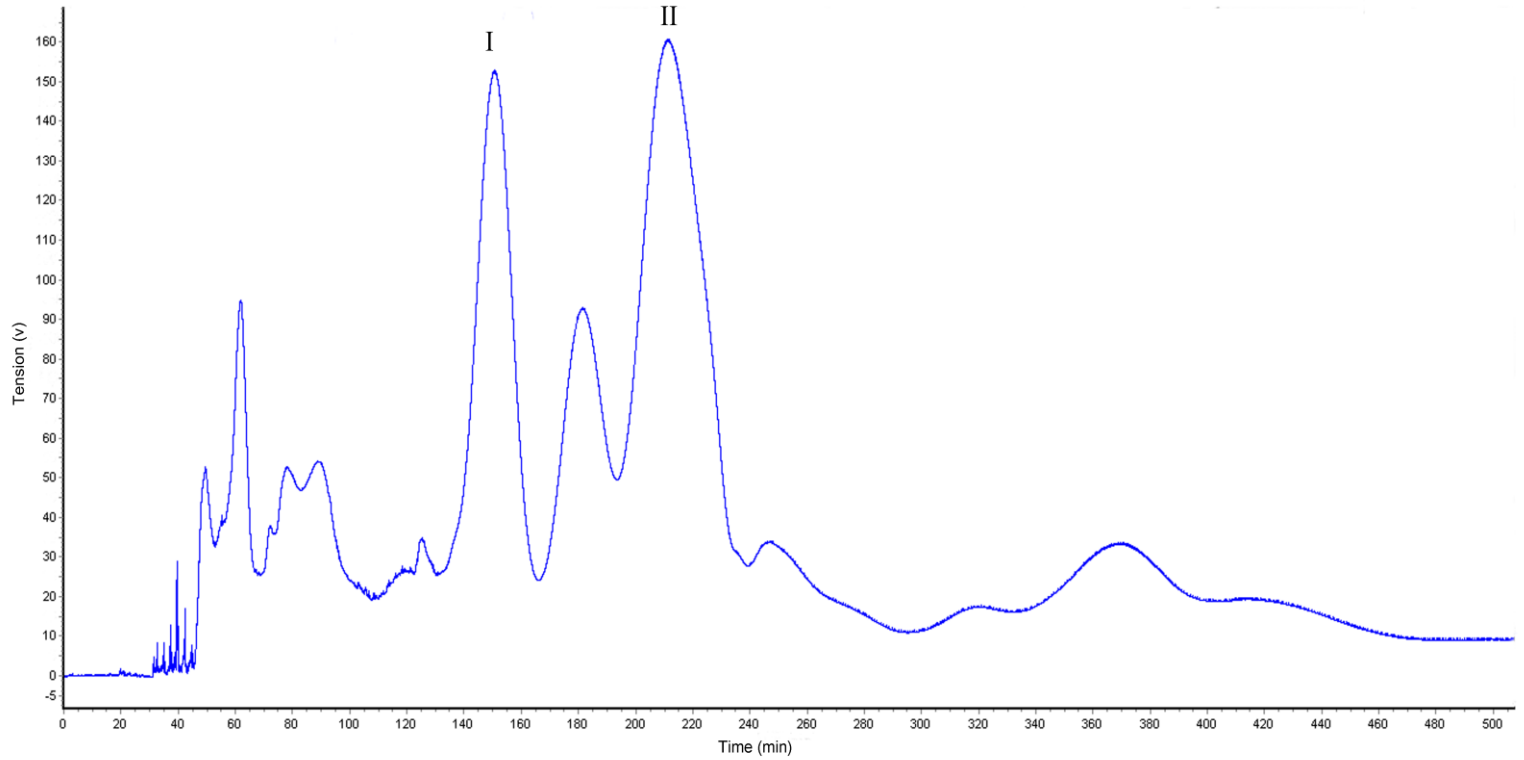

Figure 3. HSCCC chromatogram of crude extract from *C. aculeatus Merr.* Two-phase solvent system, n-hexane-ethyl acetate-methanol-water (2.3:2:2:1.3 v/v); mobile phase, the lower phase; flow rate, 2.0 mL/min; revolution speed, 900rpm; detection wavelength, 254 nm; separation temperature, 20°C; sample size, 250 mg crude sample dissolved in 10 mL of the upper phase and 10 ml of the lower phase. Retention of the stationary phase: 67%

ACKNOWLEDGEMENTS

This project was supported by the National Centerfor Complementary and Alternative Medicine of NIH (No. FO5AT002013-03) and was partly supported by Fund for Social Development Plan Projects of Technology Department of Guang dong province, No. 2006B35604001, Guangzhou Technology Projects, No.2007JC0081. The authors would like to thank South China Agricultural University for the measurement of infrared (IR), NMR spectra and EI-MS.

REFERENCES

Guo YQ, Li X, Xu J, Li N, Meng DL, Wang JH (2004) . Sesquiterpene esters from the fruits of *Celastrus orbiculatus*. Chem. Pharm. Bull. (Tokyo), 2: 1134-1136.

Ito Y (1981). Efficient preparative counter-current chromatography with a coil planet centrifuge.Journal of Chromatogr. A, 214(1): 122-125.

Ito Y (2003).Technical guidance for high-speed counter-current chromatography, Foods Food Ingredients J. Jpn., 208(9): 709-724.

Ito Y (2005). Golden rules and pitfalls in selecting optimum conditions for high-speed counter-current chromatography. J. Chromatogr. A, 065(2): 145-168.

Jin HZ, Hwang BY, Kim HS, Lee JH, Kim YH, Lee JJ (2002) . Antiinflammatory constituents of *Celastrus orbiculatus* inhibit the NF-kappaB activation and NO production. J. Nat. Prod., 65: 89-91.

Jiang Yi, Li He, Luo SQ (1996). Progress of studies on effective composition from *Celastrus orbiculatus Thunb*. Chin.

Herbal Med., 27(2): 73-75.
Kim SE, Kim YH, Lee JJ, Kim YC (1998). A new sesquiterpene ester from Celastrus orbiculatus reversing multidrug resistance in cancer cells. J. Nat. Prod., 61: 108-111.
Masahiro T, Jun K, Takashi Y, Tomoyuki O, Yusuke M (2010). Syntheses and antibacterial activities of diterpene catechol derivatives with abietane, totarane and podocarpane skeletons against methicillin-resistant Staphylococcus aureus and Propionibacterium acnes. Chem. Pharm. Bull., 58: 818-824.
Majumder PL, Maiti DC, Kraus W, Bokel M (1987). Nimbidiol,a modidied diterpenoid of the root-bark of Azadirachta indica. Phytochemistry, 6(11): 3021-3023.
Yang PM, Xiao JY, Ouyang DW, Ni X, Xu HY, Chen J (2009). Method for preparing high-purity pristimerin by high-speed countercurrent chromatography. CN 101519420 (A).
Westerheide SD, Bosman JD, Mbadugha BN, Kawahara TL, Matsumoto G, Kim S, Gu W, Devlin JP, Silverman RB, Morimoto RI (2004). Celastrols as inducers of the heat shock response and cytoprotection. J. Biol. Chem., 279: 5605-5606.