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鸦胆子果实的化学成分研究

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摘要：为了解鸦胆子(*Brucea javanica*)的化学成分，从鸦胆子果实中分离得到 13 个已知化合物，经波谱学分析鉴定为：对羟基苯甲醛 (**1**)，对羟基苯甲酸 (**2**)，3,4-二羟基苯甲酸 (**3**)，3,4-二羟基苯甲酸甲酯 (**4**)，没食子酸 (**5**)，丁香酸 (**6**)，二氢阿魏酸 (**7**)，毛地黄黄酮 (**8**)，angophorol (**9**)，2β,6β,9β-trihydroxyclovane (**10**)，硬脂酸 (**11**)，β-谷甾醇 (**12**)和 β-胡萝卜苷 (**13**)。化合物 **2**, **4**, **6** ～ **10** 均系从鸦胆子果实中首次分离得到。

关键词：鸦胆子；酚性化合物；倍半萜；脂肪酸
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Chemical Constituents of Fruits from *Brucea javanica*

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Abstract: The aim was to understand the chemical constituents of *Brucea javanica*, thirteen known compounds were isolated from the fruits of *B. javanica* by using solvent fractionation and column chromatography. On the basis of spectral data, they were identified as para-hydroxybenzaldehyde (**1**), *para*-hydroxybenzoic acid (**2**), 3,4-dihydroxybenzoic acid (**3**), methyl 3,4-dihydroxybenzoate (**4**), gallic acid (**5**), syringic acid (**6**), dihydroferulic acid (**7**), luteolin (**8**), angophorol (**9**), 2β,6β,9β-trihydroxyclovane (**10**), stearic acid (**11**), β-sitosterol (**12**), β-daucosterol (**13**). The compounds **2**, **4**, and **6** – **10** were obtained from the fruits of *B. javanica* for the first time.
Key words: *Brucea javanica*; Phenolic compound; Sesquiterpene; Fatty acid

The genus *Brucea* of the family Simaroubaceae comprises *ca*. 6 species, mainly distributed in Africa, tropical regions of Asia and northern Oceania, only with two spices (*B. mollis* and *B. javanica*) in China[1]. Its characteristic components are quassinoids[2], which possess various biological activities including anti-tumor[3], anthelmintic[4–7], anti-viral[8], anti-bacterial[9] and hyperglycemic[10] activities. *Brucea javanica* (L.) Merr., called 'Yadanzi', distributes in south of China (mainly Guangxi and Guangdong Provinces) and shows significantly antitumor and other activity mostly due to quassinoids, triterpenoids and alkaloids[2]. As a continuation of our search for naturally occurring bioactive substances from herb medicine in China, we investigated the constituents of the air-dried fruits of *B. javanica* purchased from Qingping Traditional Chinese Medicine Market and isolated a series of structurally diverse compounds, including nine phenolic constituents (**1** – **9**), two sterides (**12** – **13**), one sesquiterpenes (**10**), one fatty acid (**11**).

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1 Experiment

1.1 Instrument

Optical rotations were measured on a Perkin-Elmer 343 Polarimeter. NMR spectra were recorded on a Bruker DRX-400 spectrometer using solvent residual peaks (CD_3OD: δ_H 3.31 and δ_C 49.0 ppm; DMSO: δ_H 2.50 and δ_C 39.52 ppm; $CDCl_3$: δ_H 7.26 and δ_C 77.16 ppm) as references. ESIMS was taken on a MDS SCIEX API 2000 LC/MS/MS apparatus. For column chromatography, silica gel (200 – 300 mesh, Qingdao Haiyang Chemical Co. Ltd., Shandong, China), and MCI gel (75 – 150 μm, Mitsubishi Chemical Co. Ltd., Japan) were used. TLC was performed on precoated silica gel HSGF254 plates (Yantai Jiangyou Silica Gel Development Co. Ltd., Shandong, China) and RP-18 F254S plates (Merck Japan Ltd., Tokyo, Japan), and the spots were detected first under UV light (λ = 254 and 365 nm, respectively), and then by spraying 10% H_2SO_4 in EtOH and heating.

1.2 Plant material

Air-dried mature fruits of *Brucea javanica* were purchased from Qingping Traditional Chinese Medicine Market, Guangzhou, China, in January 2008. They were botanically authenticated by Prof. Yun-fei DENG of South China Botanical Garden, Chinese Academy of Sciences (SCGB), and a voucher specimen (No. MZH0173) was deposited in the Laboratory of SCBG, Guangzhou.

1.3 Extraction and isolation

The air-dried fruits (10 kg) were powdered and extracted three times with 95% ethanol (50 L, each) at room temperature. The pooled solvents were evaporated *in vacuo* to give a crude extract (*ca*. 2 L), which was defatted with petroleum ether and then sequentially fractionated with EtOAc and *n*-BuOH to yield EtOAc-soluble (200 g) and *n*-BuOH-soluble (185 g) fractions, respectively.

The petroleum ether-soluble extract was subjected to silica gel column chromatography (CC) and eluted with gradient mixtures of petroleum ether-ethyl acetate (1∶0 – 0∶1, V/V) to afford fractions P1 – P8. Compounds **11** (167 mg) and **12** (632 mg) were obtained by recrystallization from P3 and P5, respectively. The EtOAc-soluble fraction was subjected to silica gel CC eluted with $CHCl_3$-MeOH (10∶0 – 4∶6, V/V) to afford fractions E1 – E10. Fraction E4 was separated by Sephadex LH-20 CC eluted with MeOH followed by silica gel CC to yield compounds **1** (41 mg) and **4** (70 mg). Compound **13** (765 mg) was obtained by

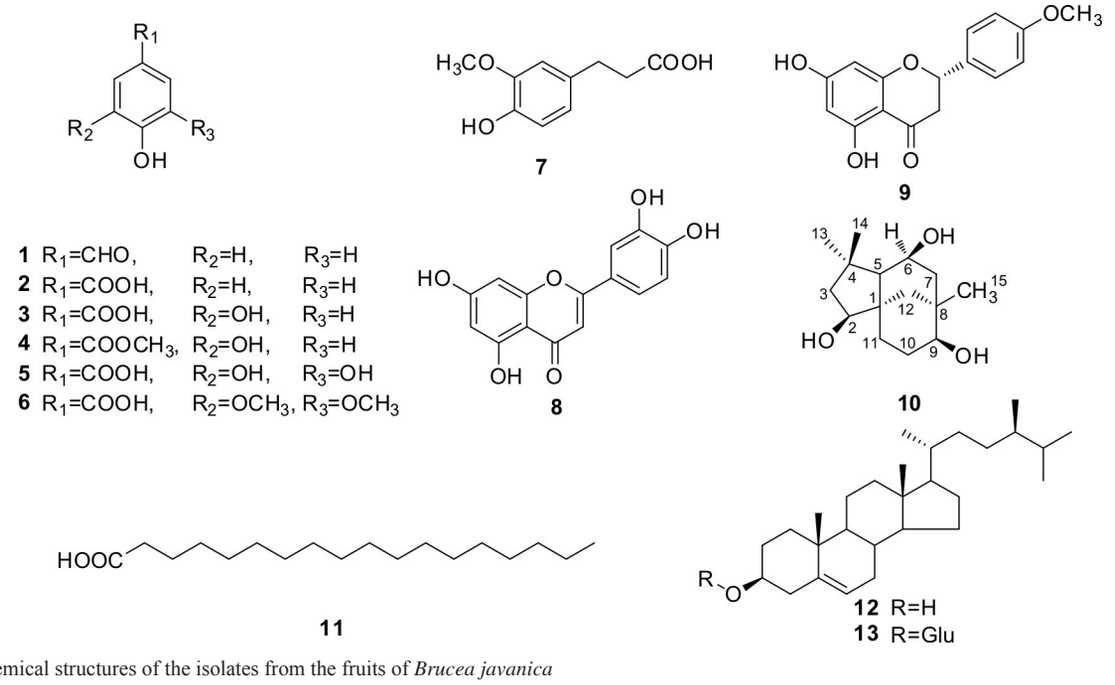

Fig.1 Chemical structures of the isolates from the fruits of *Brucea javanica*

recrystallization from Fraction E5 in $CHCl_3$/MeOH and the mother liquor was subjected to silica gel CC eluted with $CHCl_3$-MeOH (95 ∶ 5 – 80 ∶ 20, V/V) to furnish fractions E51 – E57. Fraction E53 was purified by Sephadex LH-20 CC eluted with MeOH to give compounds **6** (16 mg) and **7** (8 mg). Fraction E7 was purified by silica gel CC to give compound **2** (430.8 mg). Fraction E9 was passed through a MCI column for depigmentation, and the resultant MeOH elution was further separated by repetitive CC over silica gel and Sephadex LH-20 and purified by HPLC with MeOH-H_2O as a mobile phase to furnish compounds **10** (12 mg) and **9** (5 mg). The *n*-BuOH-soluble fraction was subjected to silica gel CC to yield fractions B1 – B8. Fraction B4 was separated by Sephadex LH-20 CC, silica gel CC and preparative TLC to afford compound **8** (36 mg).

2 Structure identification

***para*-Hydroxybenzaldehyde (1)**[11] $C_7H_6O_2$; colorless needles; ESIMS *m*/*z* (%): 122 (M^+, 100); ^{1}H NMR (400 MHz, CD_3OD): δ 9.76 (1H, s, CHO), 7.80 (2H, d, *J* = 8.4, H-2, 6), 6.91 (2H, d, *J* = 8.5 Hz, H-3, 5).

***para*-Hydroxybenzoic acid (2)**[12] $C_7H_6O_3$; white amorphous powder; ESIMS *m*/*z* (%): 138 (M^+, 100); ^{1}H NMR (400 MHz, DMSO): δ 12.42 (1H, s, COOH), 10.23 (1H, s, OH), 7.81 – 7.76 (2H, m, H-2, 6), 6.84 – 6.80 (2H, m, H-3, 5); ^{13}C NMR (100 MHz, DMSO): δ 167.2 (s, -COOH), 161.6 (s, C-1), 131.6 (d × 2, C-2, 6), 121.4 (d × 2, C-3, 5), 115.2 (s, C-4).

3,4-Dihydroxybenzoic acid (3)[12] $C_7H_6O_4$; colorless needles; ESIMS *m*/*z* (%): 146 (M^+, 100); ^{1}H NMR (400 MHz, CD_3OD): δ 7.42 – 7.36 (2H, m, H-2, 6), 6.80 – 6.73 (1H, d, *J* = 8.1 Hz, H-5); ^{13}C NMR (100 MHz, CD_3OD): δ 170.2 (s, -COOH), 151.5 (s, C-4), 146.0 (s, C-3), 123.9 (d, C-6), 123.1 (s, C-1), 117.7 (d, C-5), 115.7 (d, C-2).

Methyl 3,4-dihydroxybenzoate (4)[13] $C_8H_8O_3$; colorless needles; ESIMS *m*/*z* (%): 168 (M^+, 100); ^{1}H NMR (400 MHz, CD3OD): δ 7.59 – 7.53 (2H, m, H-2, 6), 6.87 – 6.80 (1H, d, *J* = 8.0 Hz, H-5), 3.93 (3H, s, -OMe); ^{13}C NMR (100 MHz, CD3OD): δ 170.2 (s, -C=O), 152.6 (s, C-4), 148.6 (s, C-3), 125.3 (d, C-6), 123.1 (s, C-1), 115.9 (d, C-2), 113.8 (d, C-5), 56.4 (q, -OMe).

Gallic acid (5)[12] $C_7H_6O_5$; colorless needles; ESIMS *m*/*z* (%): 170 (M^+, 100); ^{1}H NMR (400 MHz, DMSO): δ 12.22 (1H, s, COOH), 9.04 (3H, m, OH-3, 4, 5), 6.98 – 6.73 (2H, s, H-2, 6); ^{13}C NMR (100 MHz, DMSO): δ 167.5 (s, COOH), 145.4 (s × 2, C-3, 5), 138.0 (s, C-4), 120.5 (s, C-1), 108.7 (d × 2, C-2, 6).

Syringic acid (6)[14] $C_9H_{10}O_5$; colorless needles; ESIMS *m*/*z* (%): 198 (M^+, 100); ^{1}H NMR (400 MHz, CD_3OD): δ 7.33 (2H, s, H-2, 6), 3.88 (6H, s, OMe-3,5); ^{13}C NMR (100 MHz, CD3OD): δ 170.0 (s, -COOH), 148.8 (s × 2, C-3, 5), 141.7 (s, C-4), 121.9 (s, C-1), 108.3 (d × 2, C-2, 6), 56.8 (q × 2, 3, 5-OMe).

Dihydroferulic acid (7)[15] $C_9H_{10}O_4$; white amorphous powder, ESIMS *m*/*z* (%): 196 (M^+, 100); ^{1}H NMR (400 MHz, DMSO): δ 12.10 (1H, s, COOH), 8.71 (1H, s, OH), 6.78 (1H, d, *J* = 1.7 Hz, H-2), 6.65 (1H, d, *J* = 8.0 Hz, H-5), 6.58 (1H, dd, *J* = 8.0, 1.7 Hz, H-6), 3.73 (3H, s, 3-OMe), 2.70 (2H, t, *J* = 7.7 Hz, H-1′), 2.49 (2H, m, H-2′).

Luteolin (8)[16] $C_{15}H_{10}O_6$; yellow amorphous powder; ESIMS *m*/*z* (%): 286 (M^+, 100); ^{1}H NMR (400 MHz, DMSO): δ 12.98 (s, 1H, 5-OH), 7.43 (1H, d, *J* = 2.2 Hz, H-2′), 7.40 (2H, s, H-6′), 6.89 (1H, d, *J* = 8.1 Hz, H-5′), 6.67 (1H, s, H-3), 6.45 (1H, d, *J* = 2.0 Hz, H-8), 6.19 (1H, d, *J* = 2.0 Hz, H-6); ^{13}C NMR (100 MHz, DMSO): δ 181.6 (s, C-4), 164.2 (s, C-7), 163.9 (s, C-2), 161.4 (s, C-5), 157.3 (s, C-9), 149.7 (s, C-4′), 145.7 (s, C-3′), 121.4 (s, C-1′), 118.9 (d, C-6′), 116.0 (d, C-5′), 113.3 (d, C-2′), 103.6 (s, C-10), 102.8 (d, C-3), 98.8 (d, C-6), 93.8 (d, C-8).

Angophorol (9)[17] $C_{16}H_{14}O_5$; white amorphous powder; ESIMS *m*/*z* (%): 286 (M^+, 100); ^{1}H NMR (400 MHz, C_5D_5N): δ 12.32 (1H, s, 5-OH), 11.53 (1H, s, 7-OH), 7.20 (2H, m, H-2′, 6′), 6.92 (2H, m, H-3′, 5′), 5.99 (1H, d, *J* = 2.2 Hz, H-8), 5.90 (1H, d, *J* = 2.2 Hz, H-6), 5.15 (1H, dd, *J* = 12.9, 2.8 Hz, H-2), 2.95 (1H, dd, *J* = 17.1, 12.9 Hz, H-3ax), 2.56 (1H, dd, *J* = 17.1, 3.0 Hz, H-3eq); ^{13}C NMR (100 MHz, C_5D_5N): δ 197.5 (s, C-4), 168.7 (s, C-7), 165.1 (s, C-5), 164.1

(s, C-9), 160.1 (s, C-4′), 130.1 (s, C-1′), 129.3 (d × 2, C-2′, 6′), 116.9 (d × 2, C-3′, 5′), 104.2 (s, C-10), 95.9 (d, C-8), 94.9 (d, C-6), 80.2 (d, C-2), 56.2 (q, 4′-OMe), 43.7 (t, C-3).

2β,6β,9β-Trihydroxyclovane (10)[18] $C_{15}H_{26}O_3$; colorless needles; ESIMS *m*/*z* (%): 256 (M^+, 100); ^{1}H NMR (400 MHz, CD_3OD): δ 3.85 – 3.71 (2H, m, H-2, 6), 3.26 (1H, s, H-9), 2.11 – 1.99 (1H, m, H-10α), 1.72 (1H, dd, *J* = 13.4, 4.6 Hz, H-10β), 1.69 – 1.63 (1H, m, H-7α), 1.62 – 1.54 (4H, m, H-7β, 3α, 3β, 11α), 1.35 (1H, d, *J* = 11.2 Hz, H-5), 1.16 (3H, s, 14-CH_3), 1.09 (2H, m, H-12A, 11β), 1.03 (3H, s, 15-CH_3), 0.98 (3H, s, 13-CH_3), 0.83 (1H, d, *J* = 12.8 Hz, H-12B); ^{13}C NMR (100 MHz, CD_3OD): δ 80.8 (d, C-2), 75.6 (d, C-9), 69.1 (d, C-6), 57.6 (d, C-5), 48.6 (t, C-3), 46.7 (s, C-1), 44.3 (t, C-12), 37.5 (s × 2, C-4, 8), 36.6 (t, C-7), 33.1 (q, C-14), 29.0 (q, C-15), 27.8 (t, C-11), 26.6 (t, C-10), 24.7 (q, C-13).

Stearic acid (11)[12] $C_{18}H_{36}O_2$; white amorphous powder; ESIMS *m*/*z* (%): 284 (M^+, 100); ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.32 (s, 1H, 5-OH), 11.53 (s, 1H, 7-OH), 11.20 (1H, br s, COOH), 2.35 (2H, t, *J* = 7.6 Hz, H-17), 5.99 (2H, m, H-16), 1.25 (28H, m, H-15 ~ 2), 0.88 (1H, t, *J* = 6.8 Hz, CH_3-1).

3 Results and discussion

The phytochemical study on the fruits of *Brucea javanica* have led to the isolation of 13 structurally diversified compounds, through an analysis of spectral data (EI-MS, NMR), which be identified as *para*-hydroxybenzaldehyde (**1**), *para*-hydroxybenzoic acid (**2**), 3,4-dihydroxybenzoic acid (**3**), methyl 3,4-dihydroxybenzoate (**4**), gallic acid (**5**), syringic acid (**6**), dihydroferulic acid (**7**), luteolin (**8**), angophorol (**9**), 2β,6β,9β-trihydroxyclovane (**10**), stearic acid (**11**), β-sitosterol (**12**), β-daucosterol (**13**) (compounds **12** and **13** were identified by direct TLC comparison with authentic samples). The compounds (**2**, **4**, **6** – **10**) were obtained from the fruits of *B. javanica* for the first time.

According to the literatures, the compounds isolated in this study were shown to have broad-spectrum biological activities, with emphasis on the phenolic acids for their *in vitro* antiviral, antibacterial, and antifungal activities and cytotoxicity[19]. *Para*-hydroxybenzaldehyde (**1**) and *para*-hydroxybenzoic acid (**2**) derivatives were reported as antibacterial agents[20–21]. Luteolin (**8**) was determined to be an emerging anti-cancer and anti-inflammation flavonoid[22–23]. Scientific knowledge on quassinoids and the biological properties of *B. javanica* has accumulated rapidly in recent years, however, less progress had made on the presence and biological activities on other components rather than quassinoids. Our current investigation revealed the existence of many other constituents in addition to quassinoids, whose bioactivities may account for the diversified bioactivities and the traditional medical use of *B. javanica* passed through generations. Moreover, these chemical entities from the fruits of *B. javanica* could certainly play an important role in the discovery of new and effective therapeutic agents.

References

[1] Wu Z Y, Raven P H, Hong D Y. Floral of China, Vol. 11 [M]. Beijing: Science Press & St. Louis: Missouri Botanical Garden Press, 2008: 103–104.

[2] Liu J H, Jin H Z, Zhang W D, et al. Chemical constituents of plants from the genus *Brucea* [J]. Chem Biodiv, 2009, 6(1): 57–70.

[3] Fiaschetti G, Grotzer M A, Shalaby T, et al. Quassinoids: From traditional drugs to new cancer therapeutics [J]. Curr Med Chem, 2011, 18(3): 316–328.

[4] Elkhateeb A, Tosa Y, Matsuura H, et al. Antitrypanosomal activities of acetylated bruceines A and C: A structure-activity relationship study [J]. J Nat Med, 2012, 66(1): 233–240.

[5] Elkhateeb A, Yamasaki M, Maede Y, et al. Anti-babesial quassinoids from the fruits of *Brucea javanica* [J]. Nat Prod Commun, 2008, 3(2): 145–148.

[6] Gillin F D, Reiner D S, Suffness M. Bruceantin: A potent amoebicide from a plant, *Brucea antidysenterica* [J]. Antimicrob Agents Chem, 1982, 22(2): 342–345.

[7] Wang Y, Wu Z F, Wang G X, et al. In vivo anthelmintic activity of bruceine A and bruceine D from *Brucea javanica* against *Dactylogyrus intermedius* (Monogenea) in goldfish (*Carassius auratus*) [J]. Vet Parasitol, 2011, 177(1/2): 127–133.

[8] Yan X H, Chen J, Di Y T, et al. Anti-tobacco mosaic virus (TMV)

quassinoids from *Brucea javanica* (L.) Merr. [J]. J Agric Food Chem, 2010, 58(3): 1572–1577.

[9] Rahman S, Fukamiya N, Okano M, et al. Anti-tuberculosis activity of quassinoids [J]. Chem Pharm Bull, 1997, 45(9): 1527–1529.

[10] Noorshahida A, Wong T W, Choo C Y. Hypoglycemic effect of quassinoids from *Brucea javanica* (L.) Merr. (Simaroubaceae) seeds [J]. J Ethnopharmacol, 2009, 124(3): 586–591.

[11] Shi X F, Bai Z H, Liu D Y, et al. Study on the chemical constituent from the dichloromethane extract of the pine needles of *Cedrus deodara* [J]. J Chin Med Mat, 2012, 35(3): 404–406. (in Chinese)

[12] Wang Y Y, Chen H D, Liao M, et al. Studies on the chemical constituents of *Sarcopyramis nepalensis* [J]. J Chin Med Mat, 2009, 32(9): 1395–1397.(in Chinese)

[13] Tsuda T, Watanabe M, Ohshima K, et al. Antioxidative components isolated from the seed of tamarind (*Tamarindus indica* L.) [J]. J Agric Food Chem, 1994, 42(12): 2671–2674.

[14] Chen C C, Yu H J, Ou J C, et al. Constituents of the heartwood of *Diospyros eriantha* [J]. J Chin Chem Soc Taipei, 1994, 41(2): 195–198.

[15] Sun B H, Yashikawa, Chen Y J, et al. Chemistry study of *Stellaria dichatoma* L. var. *lanceolata* Bge [J]. J Shenyang Pharmaceut Univ, 2006, 23(2): 84–87.

[16] Chen F Z, Xiang Q X, Li S H. Chemical constituents in the leaves of *Alsophila spinulosa* [J]. Acta Bot Boreali-Occid Sin, 2008, 28(6): 1246–1249.

[17] Wagner H, Chari V M, Sonnenbichler J. ^{13}C-NMR-spektren naturlich vorkommender flavonoide [J]. Tetrahed Lett, 1976, 17(21): 1799–1802.

[18] Arantes S F, Hanson J R, Mobbs D J. The microbiological hydroxylation of some 2,9-dioxygenated clovanes by *Mucor plumbeus* [J]. Phytochemistry, 1999, 52(4): 631–634.

[19] Öz Elik B, Kartal M, Orhan I. Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids [J]. Pharm Biol, 2011, 49(4): 396–402.

[20] Yang S Q. Antimicrobial activity and development trend of para-hydroxybenzoic acid esters derivatives [J]. Beverage Fast Frozen Food Ind, 2004, 10(4): 25–29.

[21] Wei J, Dai H F, Yu L, et al. Antibacterial metabolites from the endophytic fungus R7 of *Aquilaria sinensis* [J]. Chin J Antibiot, 2011, 36(8): 576–580.

[22] Chen C Y, Peng W H, Tsai K D, et al. Luteolin suppresses inflammation-associated gene expression by blocking NF-κB and AP-1 activation pathway in mouse alveolar macrophages [J]. Life Sci, 2007, 81(23/24): 1602–1614.

[23] Chowdhury A R, Sharma S, Mandal S, et al. Luteolin: An emerging anti-cancer flavonoid, poisons eukaryotic DNA topoisomerase I [J]. Biochem J, 2002, 366(2): 653–661.