Short Communication

Five chemical constituents of the ethyl acetate fraction from ethanol extract of semen litchi

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Five chemical constituents were isolated from the ethyl acetate portion from ethanol extract of semen litchi (Seed of Litchi chinensis) by column chromatography on silica gel and Sephadex LH-20 coupled with preparative silica gel thin layer chromatography (TLC). The chemical structures were elucidated on the basis of physicochemical properties and spectroscopic data as stigmasterol (1), P-hydroxy-benzaldehyde (2), protocatechuic acid (3), daucosterol (4) and kaempferol-3-O-β-D-glucopyranoside (5). Compounds 2 and 5 were obtained from seed of L. chinensis for the first time.

Key words: Litchi chinensis, semen litchi, seed of Litchi chinensis, P-hydroxy-benzaldehyde, kaempferol-3-O-β-D-glucopyranoside.

INTRODUCTION

Semen litchi, the dry mature seed of Litchi chinensis Sonn which belongs to Sapindaceae, is widely distributed in the South of China and recorded in Chinese Pharmacopoeia. Evidences demonstrated that semen litchi and its ethanol extract have many pharmacologic effects including blood glucose and blood lipid modulation, anti-oxidation, anti-virus, anti-tumor, anti-liver injury, as well as enhancing insulin sensitivity (Guo et al., 2006). The enol compounds, flavonoids and total saponins were isolated from ethanol extract of seed of L. chinensis (Le and Fu, 2001; Yan and Liu, 2009; Tu et al., 2002).

MATERIALS AND METHODS

Plant material

Semen litchi in this experiment was purchased from Guangzhou Drug Co., Ltd. and was identified as the dry mature seed of L. chinensis Sonn by Professor Guangqing Qiu in Guangdong Provincial Institute of Materia Medica. A voucher specimen (No. 100501) is deposited in Guangzhou Hospital of Traditional Chinese Medicine.

Experimental material

NMR spectra were measured on a Bruker AV-600 NMR spectrometer. Column chromatography was carried out on silica gel (160 to 200 mesh and 200 to 300 mesh) and GF²⁵⁴ Silica gel that were products of Qingdao Marine Chemistry Co. Ltd. Sephadex LH-20 was the product of Sweden Pharmacia biotech. Other reagents were of analytical grade.

Extraction and isolation

2 kg of seed of L. chinensis was crushed into powder and ultrasonic extracted with 10 L of 95% ethanol for 3 times. The ethanol extract solution was filtered and concentrated under reduced pressure to give 55 g of extract. The extract (45 g) was suspended in water and then sequentially extracted with petroleum ether and ethyl acetate. The extracts were evaporated under reduced pressure to obtain 3 g of petroleum ether extract, 16 g of ethyl acetate extract and 21 g of water extract.

The ethyl acetate extract (15 g) was subjected to silica gel column chromatography and eluted gradiently with petroleum ether-ethyl acetate (100:0, 100:30, 100:50, 100:80, 100:100 and 0:100). Subsequently, 6 fractions (Fr1, Fr2, Fr3, Fr4, Fr5 and Fr6) were obtained. Compound 1 (25 mg) was further purified by
Results

Five chemical constituents were identified as stigmasterol (1), P-hydroxy-benzaldehyde (2), protocatechuic acid (3), daucosterol (4) and kaempferol-3-O-β-D-glucopyranoside (5), respectively, by NMR analyses, based on the following characteristics.

Compound 1 was isolated as white needles. It appeared purplish red spots when spraying with 10% concentrated sulfuric acid and ethanol solution. 1H-NMR (CDCl₃, 600 MHz) δ: 5.32 (1H, d, J = 5.2 Hz, H-6), 5.15 (1H, dd, J = 8.4, 15.0 Hz, H-22), 5.01 (1H, dd, J = 8.4, 15.0 Hz, H-23), 3.51 (1H, m, H-3), 1.02 (3H, s, Me-21), 1.01 (3H, s, Me-19), 0.84 (3H, s, Me-26), 0.81 (3H, t, Me-29), 0.79 (3H, s, Me-27), 0.69 (3H, s, Me-18); 13C-NMR (150 MHz, CDCl₃) δc: 140.8 (C-5), 138.3 (C-22), 129.3 (C-23), 121.7 (C-6), 71.8 (C-3), 56.8 (C-14), 56.1 (C-17), 56.0 (C-24), 50.2 (C-9), 42.4 (C-4), 42.3 (C-13), 40.4 (C-20), 39.8 (C-12), 37.3 (C-1), 36.6 (C-10), 32.1 (C-25), 31.9 (C-7, 8), 31.7 (C-2), 28.6 (C-16), 25.4 (C-28), 24.4 (C-15), 21.2 (C-21), 21.0 (C-11, 26), 19.4 (C-19), 19.0 (C-27), 12.2 (C-29), 12.1 (C-18). The physicochemical properties and spectroscopic data were consistent with the reference (Jin et al., 2007; Wang et al., 2010). Color reaction and Rf values on TLC were identical with stigmasterol reference. Compound 1 was identified as stigmasterol.

Compound 2 was isolated as white needles. K₂Fe(CN)₆·FeCl₃ reaction results in Prussian blue. 1H-NMR (CD₂OD, 600 MHz) δ: 9.75 (1H, s, CHO), 7.90 (2H, d, J = 9.0 Hz, H-2, 6), 6.93 (2H, d, J = 9.0 Hz, H-3, 5); 13C-NMR (CD₂OD, 150 MHz) δc: 196.2 (CHO), 162.6 (C-4), 131.5 (C-2, 6), 130.5 (C-1), 115.9 (C-3, 5). These physicochemical properties and spectroscopic data were consistent with the reference (Luo et al., 2011). Compound 2 was identified as P-hydroxy-benzaldehyde.

Compound 3 was isolated as white needles. K₂Fe(CN)₆·FeCl₃ reaction results in Prussian blue. 1H-NMR (CD₂OD, 600 MHz) δ: 7.52 (1H, d, J = 1.2 Hz, H-2), 6.89 (1H, d, J = 8.4 Hz, H-5), 7.47 (1H, dd, J = 1.2, 8.4 Hz, H-6); 13C-NMR (CD₂OD, 150 MHz) δc: 145.5 (C-3), 150.7 (C-4), 123.6 (C-1), 123.6 (C-6), 115.7 (C-5), 117.4 (C-2), 167.8 (C=O). These physicochemical properties and spectroscopic data were consistent with the reference (Feng et al., 2011). Compound 3 was identified as protocatechuic acid.

Compound 4 was isolated as white powder. It was hardly soluble both in methanol and in acetyl. It was identified as daucosterol by comparison TLC with standard control.
Compound 5 was isolated as yellow powder. When spraying and heating with 5% sulfuric acid, it showed yellow. 1H-NMR (DMSO-d6, 600 MHz) δH: 8.04 (2H, d, J = 9.0 Hz, H-2’,6’), 6.88 (2H, d, J = 9.0 Hz, H-3’,5’), 6.43 (1H, brs, H-8), 6.21 (1H, s, H-6), 5.46 (1H, d, J = 7.8 Hz, H-1’); 13C-NMR (DMSO-d6, 150 MHz) δc: 156.6 (C-2), 133.5 (C-3), 177.8 (C-4), 161.5 (C-5), 99.0 (C-6), 164.5 (C-7), 94.0 (C-8), 156.7 (C-9), 104.3 (C-10), 121.2 (C-1’), 131.2 (C-2’,6’), 115.4 (C-3’,5’), 160.3 (C-4’), 101.2 (C-1’’), 74.5 (C-2’’), 77.8 (C-3’’), 70.2 (C-4’’), 76.7 (C-5’’), 61.2 (C-6’’). These physicochemical properties and spectroscopic data were was consistent with the reference (Long et al., 2011). Compound 5 was identified as kaempferol-3-O-β-D-glucopyranoside.

DISCUSSION

Previous studies showed that compounds 1 (stigmasterol) (Tu et al., 2002), 3 (protocatechuic acid) (Yan and Liu, 2009) and 4 (daucosterol) (Tu et al., 2002) were present in Seed of L. chinensis, which have anti-oxidant, anti-virus, and anti-tumor properties. However, compounds 2 (P-hydroxy-benzaldehyde) and 5 (kaempferol-3-O-β-D-glucopyranoside) were not reported to be isolated from Seed of L. chinensis. P-hydroxy-benzaldehyde was exhibited a strong antioxidant activity (Conde et al., 2009). Our previous study suggested that the total saponins of seed of L. chinensis could decrease blood glucose (Guo et al., 2004). Therefore, kaempferol-3-O-β-D-glucopyranoside may play a role in the enhancing insulin sensitivity in type 2 diabetic rats (T2DR) with insulin resistance (IR).

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REFERENCES