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**Phenylpropanoids from the immature fruits of black nightshade (*Solanum nigrum* L.)**

**Abstract**

Black nightshade (*Solanum nigrum* L.) has been traditionally used as traditional Chinese medicine. Phytochemical investigations of the immature fruits of *S. nigrum* have been carried out and nine phenylpropanoids (**1-9**) were obtained. Their structures were elucidated on the basis of spectroscopic and chemical methods, including MS, <sup>1</sup>H and <sup>13</sup>C-NMR. They were identified as (7*S*, 8*R*)-4-[3-hydroxymethyl-5-(3-hydroxypropyl)-2,3-dihydrobenzofuran-2-yl]-2-methoxyphenol (**1**), (7*S*, 8*R*)-dihydrodehydroconiferyl alcohol (**2**), massonianoside A (**3**), butane-2,3-diol 2-O-(6-O-caffeoyl)-β-D-glucopyranoside (**4**), 4-[(6-O-(*E*)-caffeoyl)-β-D-glucopyranosyl] vanillic acid (**5**), (+)-isolaricresinol (**6**), *trans*-cinnamic acid (**7**), ferulic acid (**8**), and 4-hydroxy cinnamic acid (**9**).

**Keywords:** black nightshade, *Solanum nigrum* L., immature fruits, chemical investigation, phenylpropanoids

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## 1. INTRODUCTION

Black nightshade (*Solanum nigrum* L.) belongs to the family of Solanaceae and is widely distributed all over the world [1]. In China, it has been used as a common traditional Chinese medicine, which has the effects of clearing heat and detoxification, activating blood circulation and removing blood stasis, promoting water and swelling, and is mainly used to treat cold and fever, toothache, and cancers. Modern pharmacological studies indicated that *S. nigrum* exhibited a variety of biological activities including antiproliferative, anti-inflammatory [2,3], antiviral, hepatoprotective, and antioxidative activities [4-6].

*S. nigrum* contains steroidal saponins, steroidal alkaloid glycosides and phenolic compounds in the previous chemical studies [7-10]. Steroidal alkaloid glycosides are the main chemical components of *Solanum* species, which possess various pharmacological activities such as antiproliferative and anti-inflammatory properties [11,12]. Currently, most of the chemical investigations on *S. nigrum* were focused on the aerial parts, while the bioactive components of its unripe berries are still unclear.

In this study, the phytochemicals from the immature fruits of *S. nigrum* has been carried out and nine phenylpropanoids were obtained.

## 2. MATERIALS AND METHODS

### 2.1. General experimental procedures

MS spectra were obtained on an Acquity UPLC-Q-TOF Microsystem (Waters Co., Milford, MA). NMR spectra were taken on a Bruker Avance III 500 MHz spectrometer (Bruker, Switzerland). ODS packed column (40–60 µm, Merck KGaA, Darastadt, Germany) and column chromatography was employed on silica gel (Anhui

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Liangchen Silicon Source Material Co. Ltd, Lu'an, China). All other analytical chemicals and reagents were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

## 2.2. Plant materials

The immature fruits of *S. nigrum* were purchased from Haerbin (Heilongjiang province, China) and dried at room temperature in the shade. The voucher specimen has been deposited in the School of Pharmacy, Guangdong Pharmaceutical University, China.

## 2.3. Extraction and isolation

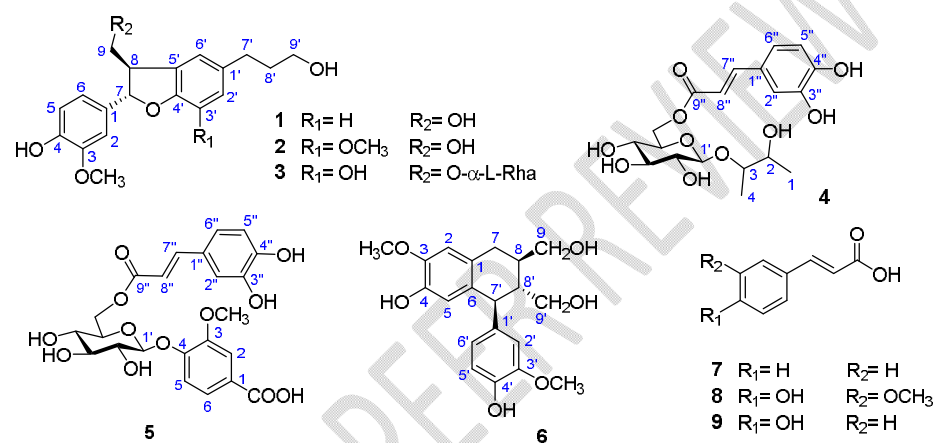
Dried immature fruits of *S. nigrum* (2.5 kg) were extracted with 70% EtOH (v/v, 15 L × 3) for 2 h. The ethanol-free suspension was subjected to a D101 macroporus resin column (80 × 1100 mm), and eluted with H<sub>2</sub>O, 10% MeOH, 30% MeOH, 50% MeOH, 70% MeOH and MeOH to give six fractions (I-VI). The 70% MeOH elution (fraction V, 51 g) was separated by a silica gel chromatography column (200-300 mesh, 1100 g) into 15 fractions (V-1 to V-15) with a CHCl<sub>3</sub>-MeOH gradient (100:1 to 0:1, v/v).

Compounds **7** (16.2 mg), **8** (10.1 mg), and **9** (16.0 mg) were obtained from fraction V-8 followed by an ODS MPLC, gradiently eluted with MeOH-H<sub>2</sub>O (1:9 to 10:0, v/v). Fraction V-6 was applied to an ODS MPLC eluted with a gradient of MeOH-H<sub>2</sub>O (1:9 to 10:0, v/v) to afford eight subfractions (V-6-1 to V-6-8). Subfraction V-6-5 was further purified by a semi-preparative HPLC to obtain compounds **1** (8.2 mg) and **3** (7.3 mg). Fraction V-7 was subjected to an ODS MPLC and eluted with MeOH-H<sub>2</sub>O gradient (1:9 to 10:0, v/v) to afford ten fractions (V-7-1 to V-7-10). Subfraction V-7-6 was further separated by an ODS MPLC and semi-preparative HPLC to obtain

compounds **2** (10.7 mg), **4** (6.3 mg), **5** (9.0 mg), and **6** (5.6 mg), respectively.

### 3. RESULTS AND DISCUSSION

The 70% ethanol extract of the immature fruits of *S. nigrum* was separated successively by column chromatography on D101 macroporus resin, silica gel, ODS MPLC, and preparative HPLC, to afford nine phenylpropanoids (**1-9**) (Fig. 1). Their structures were elucidated on the basis of spectroscopic data, including MS, and  $^1\text{H}$  and  $^{13}\text{C}$ -NMR.



**Fig. 1** Structures of the phenylpropanoids **1-9**

Compound **1**, brownish oil, was a blue fluorescence under 365 nm after TLC development. Its molecular formula was determined as  $\text{C}_{19}\text{H}_{22}\text{O}_5$  based on its ESI-MS with the ion  $m/z$  353  $[\text{M}+\text{Na}]^+$ ,  $^1\text{H}$  and  $^{13}\text{C}$ -NMR.

In the  $^1\text{H}$ -NMR, protons at  $\delta_{\text{H}}$  7.01 (1H, d,  $J=1.8$  Hz, H-2), 6.87 (1H, dd,  $J=8.2, 1.8$  Hz, H-6) and 6.80 (1H, d,  $J=8.2$  Hz, H-5) consisted an ABX coupling system of in a 1,3,4-trisubstituted benzene ring. Signals at  $\delta_{\text{H}}$  7.13 (1H, s, H-2'), 7.00 (1H, d,  $J=8.1$  Hz, H-6') and 6.70 (1H, d,  $J=8.1$  Hz, H-5') came from another ABX coupling system benzene ring in the molecule.  $\delta_{\text{H}}$  5.48 (1H, d,  $J=6.4$  Hz, H-7) was a proton linked to oxygenated carbon.  $\delta_{\text{H}}$  3.80 (3H, s, 3-OCH<sub>3</sub>) was a methoxy group.

$^{13}\text{C}$ -NMR gave 19 carbon signals (Table 1). In aromatic region ( $\delta_{\text{C}}$  159.1 to 109.5),

there was 12 carbon signals, which were two benzene ring units. Carbon signal at  $\delta_C$  56.4 in high field region is a methoxycarbon signal, which was consistent with the information given by  $^1\text{H-NMR}$ .  $\delta_C$  87.8, 54.8 and 64.8 were deduced as the carbon signals on the furan ring, and  $\delta_C$  36.1, 32.4 and 61.9 were from a hydroxypropyl group. Taken together, compound **1** was speculated a benzodihydrofuran lignin. Based on above analysis and the literature [13], compound **1** was identified as (7*S*, 8*R*)-4-[3-hydroxymethyl-5-(3-hydroxypropyl)-2,3-dihydrobenzofuran-2-yl]-2-methoxyphenol.

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The molecular formula of compound **2** was determined as  $\text{C}_{20}\text{H}_{24}\text{O}_6$  based on its ESI-MS with the ion  $m/z$  383  $[\text{M}+\text{Na}]^+$ ,  $^1\text{H}$  and  $^{13}\text{C-NMR}$ . In the  $^1\text{H-NMR}$ , protons at  $\delta_H$  7.03 (1H, d,  $J=1.8$  Hz, H-2), 6.88 (1H, dd,  $J=8.1, 1.8$  Hz, H-6) and 6.81 (1H, d,  $J=8.1$  Hz, H-5) consisted an ABX coupling system of in a 1,3,4-trisubstituted benzene ring. Signals at  $\delta_H$  6.74 (1H, s, H-2') and 6.76 (1H, d,  $J=8.1$  Hz, H-6') came from another benzene ring in the molecule.  $\delta_H$  5.50 (1H, d,  $J=6.4$  Hz, H-7) was a proton linked to oxygenated carbon.  $\delta_H$  3.82 (3H, s, 3-OCH<sub>3</sub>) and 3.81 (3H, s, 3'-OCH<sub>3</sub>) were two methoxy groups.

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In the  $^{13}\text{C-NMR}$  (Table 1), there were 20 carbon signals, including 12 carbon signals in aromatic region ( $\delta_C$  148.4 to 110.6), which were two benzene ring units. Carbon signals at  $\delta_C$  56.4 and 56.3 in high field region were two methoxy carbons, which were consistent with the information given by  $^1\text{H-NMR}$ .  $\delta_C$  88.3, 55.2 and 64.8 were deduced as the carbon signals on the furan ring, and  $\delta_C$  36.1, 32.8 and 61.8 were from a hydroxypropyl group. Therefore, compound **2** was speculated a benzodihydrofuran lignin. Based on above analysis and the literature [14], compound **2** was identified as (7*S*, 8*R*)-dihydrodehydroconiferyl alcohol.

**Table 1**  $^{13}\text{C}$  NMR data of compounds **1-6** ( $\delta$  in ppm and  $J$  in Hz)

NO.	<b>1</b> <sup>a</sup>	<b>2</b> <sup>a</sup>	<b>3</b> <sup>a</sup>	<b>4</b> <sup>b</sup>	<b>5</b> <sup>b</sup>	<b>6</b> <sup>a</sup>
1	134.9	134.8	132.6	15.1	123.7	128.5
2	110.5	110.6	110.2	78.6	112.7	111.8
3	148.5	147.4	146.4	68.9	149.1	146.5
4	147.2	148.4	147.7	19.5	151.6	145.3
5	115.6	115.7	114.8		114.9	116.9
6	119.6	119.6	118.6		123.4	138.5
7	87.8	88.3	87.2		167.9	33.8
8	54.8	55.2	50.9			40.4
9	64.8	64.8	68.9			65.9
1'	135.4	130.1	128.3	101.3	100.5	133.9
2'	125.8	113.9	115.4	73.4	73.2	113.6
3'	129.1	147.2	140.8	76.6	75.8	148.3
4'	159.1	144.9	144.7	70.5	70.3	145.9
5'	109.5	136.4	135.2	73.8	73.7	115.6
6'	129.3	117.7	116.2	63.8	64.0	122.8
7'	32.4	36.1	34.8			48.1
8'	36.1	32.8	31.5			48.4
9'	61.9	61.8	60.3			62.1
1''			100.2	125.6	125.9	
2''			70.9	113.9	115.3	
3''			72.0	145.8	145.3	
4''			70.6	148.7	147.4	
5''			68.6	115.9	116.3	
6''			18.1	121.5	120.5	
7''				145.4	144.1	
8''				114.9	115.2	
9''				166.5	165.6	
3-OCH <sub>3</sub>	56.4	56.4	55.5		55.6	56.2
3'-OCH <sub>3</sub>		56.3				56.3

<sup>a</sup> 151 MHz for  $^{13}\text{C}$  NMR in Acetone- $d_6$ .<sup>b</sup> 101 MHz for  $^{13}\text{C}$  NMR in DMSO- $d_6$ .

Compound **3**, brownish oil, was a blue fluorescence under 365 nm after TLC development. Its molecular formula was determined as  $\text{C}_{25}\text{H}_{32}\text{O}_{10}$  based on its ESI-MS with the ion  $m/z$  515  $[\text{M}+\text{Na}]^+$ ,  $^1\text{H}$  and  $^{13}\text{C}$ -NMR.

In the  $^1\text{H}$ -NMR, protons at  $\delta_{\text{H}}$  6.93 (1H, d,  $J=1.3$  Hz, H-2), 6.86 (1H, dd,  $J=8.2$ , 1.3 Hz, H-6) and 6.81 (1H, d,  $J=8.2$  Hz, H-5) consisted an ABX coupling system of in

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a 1,3,4-trisubstituted benzene ring. Signals at  $\delta_{\text{H}} 6.50$  (2H, br.s, H-2', 6') came from another benzene ring in the molecule.  $\delta_{\text{H}} 5.37$  (1H, d,  $J=6.6$  Hz, H-7) was a proton linked to oxygenated carbon.  $\delta_{\text{H}} 3.76$  (3H, s, 3-OCH<sub>3</sub>) and 1.13 (3H, d,  $J=6.2$  Hz, H-6'') were a methoxy and methyl groups in the higher field.  $\delta_{\text{H}} 4.62$  (1H, d,  $J=0.7$  Hz, H-1'') was deduced as a terminal proton signal of sugar.

In the <sup>13</sup>C-NMR (Table 1), there were 25 carbon signals, including 12 carbon signals in aromatic region ( $\delta_{\text{C}} 144.7$  to 110.2), which were two benzene ring units. Carbon signal at  $\delta_{\text{C}} 55.5$  in high field region was a methoxycarbon, which was consistent with the information given by <sup>1</sup>H-NMR. Compared with the NMR data of compound 2, it is speculated that compound 3 is a benzodihydrofuran lignin with rhamnose substitution at C-9 position. Based on above analysis and the literature [15], compound 3 was identified as massonioside A.

Compound 4 was brownish oil and exhibited a blue fluorescence under 365 nm after TLC development. Its molecular formula was determined as C<sub>19</sub>H<sub>26</sub>O<sub>10</sub> based on its ESI-MS with the ion  $m/z 437$  [M+Na]<sup>+</sup>, <sup>1</sup>H and <sup>13</sup>C-NMR.

In the <sup>1</sup>H-NMR spectrum, signals at  $\delta_{\text{H}} 7.50$  (1H, d,  $J=15.6$  Hz, H-7'') and 6.26 (1H, d,  $J=15.6$  Hz, H-8'') were speculated to be the proton signals on the trans carbon-carbon double bond. The aromatic region had three proton signals at  $\delta_{\text{H}} 7.04$  (1H, s, H-2''), 6.99 (1H, d,  $J=7.8$  Hz, H-5'') and 6.76 (1H, d,  $J=7.4$  Hz, H-6''). Signal at  $\delta_{\text{H}} 4.26$  (1H, d,  $J=7.7$  Hz, H-1'') was the terminal proton signal of sugar.  $\delta_{\text{H}} 4.40\sim 2.98$  has 8 proton signals, which were proton signals on oxymethylene or oxymethylene.  $\delta_{\text{H}} 1.07$  (3H, d,  $J=6.0$  Hz, H-1) and 0.99 (3H, d,  $J=6.0$  Hz, H-4) were two methyls in the high field.

<sup>13</sup>C-NMR spectrum indicated 19 carbon signals, and  $\delta_{\text{C}} 166.5$  was an ester carbonyl in the lower field (Table 1). Signals at  $\delta_{\text{C}} 166.5$ , 148.7, 145.8, 145.4, 125.6, 121.5,

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115.9, 114.9 and 113.9 consisted of a caffeic acyl group. Meanwhile, compound **4** has a glucose unit, which the carbon signals at  $\delta_C$  101.3, 73.4, 76.6, 70.5, 73.8 and 63.8. The remaining four carbon signals at  $\delta_C$  78.6, 68.9, 19.5 and 15.1 formed a 3-hydroxy-2-butoxy group. Based on above analysis and the literature [16], compound **4** was identified as butane-2,3-diol 2-O-(6-O-caffeoyl)- $\beta$ -D-glucopyranoside.

Compound **5** was brownish oil and exhibited a blue fluorescence under 365 nm after TLC development. Its molecular formula was determined as  $C_{23}H_{24}O_{12}$  based on its ESI-MS with the ion  $m/z$  491  $[M-H]^-$ ,  $^1H$  and  $^{13}C$ -NMR.

In the  $^1H$ -NMR spectrum, signals at  $\delta_H$  7.48 (1H, d,  $J=15.7$  Hz, H-7'') and 6.36 (1H, d,  $J=15.7$  Hz, H-8'') were speculated to be the proton signals on the *trans* carbon-carbon double bond. The aromatic region had six proton signals at  $\delta_H$  7.40 (1H, dd,  $J=8.2, 1.7$  Hz, H-6), 7.38 (1H, d,  $J=1.7$  Hz, H-2), 7.37 (1H, d,  $J=1.8$  Hz, H-2''), 7.18 (1H, dd,  $J=8.2, 1.8$  Hz, H-6''), 6.84 (1H, d,  $J=8.2$  Hz, H-5) and 6.77 (1H, d,  $J=8.2$  Hz, H-5''), which speculated that the compound had two 1,3,4-trisubstituted benzene rings. Signal at  $\delta_H$  5.08 (1H, d,  $J=7.3$  Hz, H-1') was the terminal proton signal of sugar.  $\delta_H$  3.73 (3H, s, 3-OCH<sub>3</sub>) was a methoxyl group in the high field.

The  $^{13}C$ -NMR spectrum gives 23 carbon signals (Table 1). Compared with the NMR data of compound **4**, compound **5** contained a caffeic acyl segment and a glucose unit. The chemical shifts of C-1' and C-6' of the glucose shifted to the low field, which speculated that there were substituted on C-1' and C-6'. Meanwhile, compound **5** had a vanillic acid fragment with substitutions at position 4. Based on above analysis and the literature [17], compound **5** was identified as 4-[(6-O-(*E*)-caffeoyl)- $\beta$ -D-glucopyranosyl] vanillic acid.

Compound **6** was brownish oil and its molecular formula was determined as

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C<sub>20</sub>H<sub>24</sub>O<sub>6</sub> based on its ESI-MS with the ion  $m/z$  359 [M-H]<sup>-</sup>, <sup>1</sup>H and <sup>13</sup>C-NMR. In the <sup>1</sup>H-NMR spectrum, signals in aromatic region at  $\delta_H$  6.78 (1H, s, H-5'), 6.76 (1H, s, H-2'), 6.66 (1H, s, H-2) and 6.22 (1H, s, H-5) were protons on the benzene ring. In the high field,  $\delta_H$  3.78 (3H, s, 3-OCH<sub>3</sub>) and 3.81 (3H, s, 3'-OCH<sub>3</sub>) were two methoxy signals.

The <sup>13</sup>C-NMR spectrum gave 20 carbon signals (Table 1), and there had 12 carbon signals in the aromatic region, which were two benzene ring units.  $\delta_C$  56.2 and 56.3 were two methoxy groups, which were consistent with the information given by <sup>1</sup>H-NMR spectrum. The remaining six carbon signals at  $\delta_C$  65.9, 62.1, 48.4, 48.1, 40.4 and 33.78 were speculated that it was the alkyl carbon signal on the C<sub>3</sub> skeleton of phenylpropanoid. Based on above analysis and the literature [18], compound 6 was identified as (+)-isolariciresinol.

Compounds 7 to 9 were identified as *trans*-cinnamic acid (7) [19], ferulic acid (8) [20], and 4-hydroxy cinnamic acid (9) [21], respectively, based on their spectroscopic analysis.

#### 4. CONCLUSIONS

Further chemical investigation of the immature fruits of *S. nigrum* led to the isolation of nine phenylpropanoids. Their structures were elucidated on the basis of spectroscopic and chemical methods. They were identified as compound 1 was identified as (7*S*, 8*R*)-4-[3-hydroxymethyl-5-(3-hydroxypropyl)-2,3-dihydrobenzofuran-2-yl]-2-methoxyphenol, (7*S*, 8*R*)-dihydrodehydroconiferyl alcohol, massonioside A (3), butane-2,3-diol 2-O-(6-O-caffeoyl)- $\beta$ -D-glucopyranoside, 4-[(6-O-(*E*)-caffeoyl)- $\beta$ -D-glucopyranosyl] vanillic acid, (+)-isolariciresinol,

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*trans*-cinnamic acid, ferulic acid, and 4-hydroxy cinnamic acid.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

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