Antibacterial Activity of the Flavonoids from *Dalbergia odorifera* on *Ralstonia solanacearum*

Xiabo Zhao 1,2, Wenli Mei 1, Mingfu Gong 3, Wenjian Zuo 1, Hongjin Bai 2,* and Haofu Dai 1,*

1 Hainan Key Laboratory for Research and Development of Natural Products from Li Folk Medicine, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou, Hainan 571101, China
2 Xinjiang Production and Construction Corps Key Laboratory of Protection and Utilization of Biological Resources in Tarim Basin, College of Life Science, Tarim University, Alar, Xinjiang 843300, China
3 College of Chemistry and Life Science, Leshan Normal University, Leshan, Sichuan 614000, China

* Authors to whom correspondence should be addressed; E-Mails: bhj67@163.com (H.B.); hfdai2001@yahoo.com.cn (H.D.); Tel./Fax: +86-997-468-1608 (H.B.); +86-898-669-61869 (H.D.).

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**Abstract:** Phytochemical investigation on the heartwood of *Dalbergia odorifera* resulted in the isolation of nine flavonoids. Their structures were elucidated as sativanone (1), (3R)-vestitone (2), (3R)-2',3',7-trihydroxy-4'-methoxyisoflavanone (3), (3R)-4'-methoxy-2',3,7-trihydroxyisoflavanone (4), carthamidin (5), liquiritigenin (6), isoliquiritigenin (7), (3R)-vestitol (8), and sulfuretin (9) based on their spectral data. All compounds were evaluated for their inhibitory activity against *Ralstonia solanacearum*. This is the first report about anti-*R. solanacearum* activity of the compounds from *D. odorifera*.

**Keywords:** *Dalbergia odorifera*; flavonoids; antibacterial activity; anti-*Ralstonia solanacearum*
1. Introduction

*Ralstonia solanacearum*, the pathogen that is the causal agent of bacterial wilt, is one of the best-known bacterial diseases, and is found in tropical, subtropical, and some temperate regions of the World. This soilborne pathogen attacks more than 200 plant species, including many agriculturally important crops [1]. This bacterium can also be free-living as a saprophyte in water or in the soil in the absence of host plants [2]. Streptomycin is widely used in agriculture, but the overuse of it can lead to bacterial resistance [3]. Thus, it is very necessary to search for more potent anti-*R. solanacearum* compounds.

The heartwood of *Dalbergia odorifera* T. Chen, named “Jiangxiang” in Chinese traditional medicine, was used in China and Korea for the treatment of blood stagnation syndrome, ischemia, swelling, necrosis and rheumatic pain [4,5]. Previous chemical investigations on this plant have led to the isolation of flavonoids and phenolic compounds [6-8]. Some flavonoids have been reported to possess various pharmacological effects such as anti-inflammatory, antibacterial, antiplasmodial, antinephritic, neuroprotective and antioxidant activities [9-14]. During the course of our screening for anti-*R. solanacearum* agents from tropical medicinal plants, the crude ethanol extract of the heartwood of *D. odorifera* showed anti-*R. solanacearum* activity. In this paper, we described the isolation, identification and anti-*R. solanacearum* activity of compounds 1–9.

2. Results and Discussion

The compounds (Figure 1) were identified as: sativanone (1), (3R)-vestitone (2), (3R)-2',3',7-trihydroxy-4'-methoxyisoflavanone (3), (3R)-4'-methoxy-2',3,7-trihydroxyisoflavanone (4), carthamidin (5), liquiritigenin (6), isoliquiritigenin (7), (3R)-vestitol (8), and sulfuretin (9) by comparison of their spectral data with the literature.

![Figure 1. Structures of compounds 1–9.](image-url)
Compounds 1–9 were next evaluated for their inhibitory activity against *R. solanacearum* (Table 1). Among the nine flavonoids, compound 8 exhibited the strongest antibacterial activity, with an inhibition zone diameter of 16.62 mm, which was close to that of streptomycin sulfate (the positive control). Compounds 2, 6 and 7 also showed stronger antibacterial activities than the rest of compounds, with inhibition zone diameters of 11.19, 12.23, and 14.15 mm, respectively.

| Compound | *Ralstonia solanacearum* | Compound | *Ralstonia solanacearum* |
|----------|-------------------------|----------|-------------------------|
| 1        | 6.53 ± 0.05             | 6        | 12.23 ± 0.45            |
| 2        | 11.19 ± 0.15            | 7        | 14.15 ± 0.95            |
| 3        | 8.11 ± 0.14             | 8        | 16.62 ± 1.07            |
| 4        | 9.99 ± 1.25             | 9        | 9.10 ± 1.22             |
| 5        | 8.34 ± 0.16             | Streptomycin sulfate<sup>a</sup> | 16.80 ± 0.33 |

The results of diffusion method are presented as diameters of inhibition zones in mm. Each value represents mean ± SD (n = 3).<sup>a</sup> Streptomycin sulfate was used as positive control.

Compounds 1–4 belong to the isoflavanone class. Compound 1 showed lower activity than the other compounds, and this may be due to the absence of the 2'-OH group, suggesting that this 2'-OH is a favorable group for activity. Compounds 2–4 had a B-ring OH group (2' position), and 3 had a B-ring OH group (3' position), while 4 had a C-ring OH group (3 position). Lower activity of 3 compared to that of 2 seemed to be because the 3'-OH and 2'-OH formed a stable five-membered ring, which reduced the inhibition of the 2'-OH group. Compound 4 had slightly reduced inhibition compared with 2, which leads us to speculate that the 3-OH and 2'-OH formed an unstable six-membered ring. Compound 8 belong to the isoflavane class which lack the C(4)=O in the C-ring compared with 2, and its activity was higher than that of 2. The result suggests that the presence of C(4)=O will reduced the inhibitory effect.

3. Experimental

3.1. General

The NMR spectra were recorded on a Bruker AV-400 spectrometer, using TMS as an internal standard. Column chromatography was performed with silica gel (Marine Chemical Industry Factory, Qingdao, China) and Sephadex LH-20 (Merck). TLC was performed with silica gel GF254 (Marine Chemical Industry Factory, Qingdao, China) plates.

3.2. Plant Materials

The dried heartwood of *D. odorifera* was purchased from the Haikou Free Market of Agricultural Products, Hainan Province, China, in October, 2010. The specimen was identified by Professor Zheng-fu Dai of the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, where a voucher specimen (No. 20101009) has been deposited.
3.3. Extraction, Fractionation and Identification of the Flavonoids

The dried and crushed heartwood of *D. odorifera* (8.4 kg) was extracted three times with 95% ethanol (50 L) at room temperature for three weeks totally. The ethanol extract was then filtered through absorbent gauze, and the filtrate was concentrated on a rotary evaporator under reduced pressure at 50 °C to remove ethanol, resulting in a crude ethanolic extract. This was partitioned with petroleum ether, ethyl acetate and *n*-butanol. The ethyl acetate phase (477.0 g) was submitted to column chromatography (CC) over silica gel eluted with a mixture of chloroform and methanol (100:1–0:100, v/v) of increasing polarity resulting in eighteen fractions (Fr.1–Fr.18). Compound 1 (100.0 mg) obtained by recrystallization from Fr.6 (52.0 g). Repeated CC on silica gel CC eluted with CHCl3-MeOH (100:1–0, v/v) and Sephadex LH-20 (CHCl3-MeOH, 1:1, v/v), led to the isolation of compounds 2 (34.0 mg), 3 (4.0 mg), 4 (64.4 mg), 5 (5.0 mg), 6 (10.4 mg), 7 (70.0 mg) and 8 (5.0 mg) from Fr.10 (45.0 g). Fr.12 (51.3 g) was submitted to column chromatography over silica gel eluted with CHCl3-MeOH (50:1–0, v/v) and further purification with Sephadex LH-20 (95% EtOH) to afford compound 9 (7.6 mg). The physicochemical and spectrometric data of nine flavonoids were as follows:

**Sativanone** (1). White powder; C17H16O5; 1H-NMR (CD3OD), δ: 4.40 (1H, dd, *J* = 11.0, 5.5 Hz, H-2a), 4.54 (1H, d, *J* = 11.0 Hz, H-2b), 4.16 (1H, dd, *J* = 11.0, 5.5 Hz, H-3), 7.76 (1H, d, *J* = 8.7 Hz, H-5), 6.49 (1H, d, *J* = 8.7 Hz, H-6), 6.51 (1H, s, H-8), 6.33 (1H, d, *J* = 2.3 Hz, H-3′), 6.46 (1H, dd, *J* = 8.4, 2.3 Hz, H-5′), 6.97 (1H, d, *J* = 8.4 Hz, H-6′), 3.77 (3H, s, 2′-OCH3), 3.74 (3H, s, 4′-OCH3); 13C-NMR (CD3OD), δ: 70.1 (C-2), 47.5 (C-3), 192.4 (C-4), 129.9 (C-5), 101.8 (C-6), 164.2 (C-7), 98.0 (C-8), 163.6 (C-9), 113.7 (C-10), 115.3 (C-1′), 157.7 (C-2′), 109.8 (C-3′), 160.0 (C-4′), 104.0 (C-5′), 128.5 (C-6′), 54.0 (2′-OCH3), 54.2 (4′-OCH3). These data were equal to those of literature [15].

**3R)-Vestitone** (2). Yellow crystals; C16H14O5; 1H-NMR (CD3OD), δ: 4.40 (1H, dd, *J* = 11.0, 5.4 Hz, H-2a), 4.56 (1H, d, *J* = 11.0 Hz, H-2b), 4.12 (1H, dd, *J* = 11.0, 5.4 Hz, H-3), 7.74 (1H, d, *J* = 8.8 Hz, H-5), 6.48 (1H, dd, *J* = 8.8, 2.2 Hz, H-6), 6.31 (1H, d, *J* = 2.2 Hz, H-8), 6.38 (1H, d, *J* = 2.4 Hz, H-3′), 6.34 (1H, d, *J* = 8.4 Hz, H-5′), 6.88 (1H, d, *J* = 8.4 Hz, H-6′), 3.70 (3H, s, 2′-OCH3), 3.74 (3H, s, 4′-OCH3); 13C-NMR (CD3OD), δ: 72.0 (C-2), 48.7 (C-3), 194.7 (C-4), 130.4 (C-5), 117.1 (C-6), 166.4 (C-7), 103.6 (C-8), 165.8 (C-9), 115.7 (C-10), 115.8 (C-1′), 157.6 (C-2′), 102.7 (C-3′), 161.8 (C-4′), 106.0 (C-5′), 131.8 (C-6′), 55.7 (4′-OCH3). These data were identical to those reported [16-18].

**3R)-2',3',7-Trihydroxy-4'-methoxyisoflavanone** (3). White powder; C16H14O6; 1H-NMR (CD3OD), δ: 4.49 (1H, d, *J* = 5.4 Hz, H-2a), 4.63 (1H, dd, *J* = 10.8, 5.4 Hz, H-2b), 4.17 (1H, dd, *J* = 10.8, 5.4 Hz, H-3), 7.78 (1H, d, *J* = 8.7 Hz, H-5), 6.53 (1H, dd, *J* = 8.7, 2.0 Hz, H-6), 6.35 (1H, d, *J* = 2.0 Hz, H-8), 6.45 (1H, d, *J* = 8.5 Hz, H-5′), 6.51 (1H, d, *J* = 8.5 Hz, H-6′), 3.83 (3H, s, 4′-OCH3); 13C-NMR (CD3OD, 100 MHz), δ: 71.9 (C-2), 48.7 (C-3), 194.5 (C-4), 130.4 (C-5), 111.7 (C-6), 149.1 (C-7), 104.2 (C-8), 165.6 (C-9), 116.8 (C-10), 115.5 (C-1′), 145.1 (C-2′), 135.2 (C-3′), 166.2 (C-4′), 103.6 (C-5′), 120.6 (C-6′), 56.6 (4′-OCH3). These data were consistent with those reported in [19].
(3R)-4′-Methoxy-2′,3,7-trihydroxyisoflavanone (4). White crystals; $C_{16}H_{12}O_6$; $^1$H-NMR (acetone-$d_6$), $\delta$: 4.30 (1H, d, $J = 11.8$ Hz, H-2a), 4.88 (1H, d, $J = 11.8$ Hz, H-2b), 7.74 (1H, d, $J = 8.7$ Hz, H-5), 6.56 (1H, d, $J = 8.7$ Hz, H-6), 6.36 (3H, overlapped, H-3′, 5′, 8), 7.32 (1H, d, $J = 9.3$ Hz, H-6′), 3.67 (3H, s, 4′-OCH$_3$); $^{13}$C-NMR (acetone-$d_6$), $\delta$: 75.5 (C-2), 76.0 (C-3), 191.6 (C-4), 131.6 (C-5), 112.8 (C-6), 166.5 (C-7), 104.4 (C-8), 164.9 (C-9), 114.5 (C-10), 118.9 (C-1′), 158.5 (C-2′), 104.1 (C-3′), 162.9 (C-4′), 106.7 (C-5′), 129.7 (C-6′), 56.4 (4′-OCH$_3$). These data were in accordance with those reported in [9].

Carthamidin (5). White crystals; $C_{15}H_{12}O_6$; $^1$H-NMR (CD$_3$OD), $\delta$: 5.44 (1H, dd, $J = 13.0$, 2.8 Hz, H-2), 2.83 (1H, dd, $J = 17.1$, 2.8 Hz, H-3a), 3.20 (1H, dd, $J = 17.1$, 13.0 Hz, H-3b), 6.05 (1H, s, H-8), 7.42 (2H, d, $J = 8.5$ Hz, H-2′, 6′), 6.96 (2H, d, $J = 8.5$ Hz, H-3′, 5′). These data were identical to those in the literature [20].

Liquiritigenin (6). White crystals; $C_{15}H_{12}O_4$; $^1$H-NMR (CD$_3$OD), $\delta$: 5.58 (1H, dd, $J = 13.2$, 2.8 Hz, H-2), 2.95 (1H, dd, $J = 16.9$, 2.8 Hz, H-3a), 3.26 (1H, dd, $J = 16.9$, 13.2 Hz, H-3b), 7.97 (1H, d, $J = 8.7$ Hz, H-5), 6.74 (1H, dd, $J = 8.7$, 2.2 Hz, H-6), 6.62 (1H, d, $J = 2.2$ Hz, H-8), 7.53 (2H, d, $J = 8.6$ Hz, H-2′, 6′), 7.08 (2H, d, $J = 8.6$ Hz, H-3′, 5′); $^{13}$C-NMR (CD$_3$OD), $\delta$: 80.3 (C-2), 44.5(C-3), 193.0 (C-4), 129.5 (C-5), 111.5 (C-6), 166.0 (C-7), 103.6 (C-8), 164.8 (C-9), 114.4 (C-10), 130.5 (C-1′), 128.4 (C-2′, 6′), 116.1 (C-3′, 5′), 158.1 (C-4′). These data were in accordance with those reported previously [19].

Isoliquiritigenin (7). Yellow crystals; $C_{15}H_{12}O_4$; $^1$H-NMR (CD$_3$OD), $\delta$: 7.54 (3H, dd, $J = 15.4$, 6.0 Hz, H-2, 6, α), 6.88 (2H, d, $J = 8.6$ Hz, H-3, 5), 6.25 (1H, d, $J = 2.4$ Hz, H-3′), 6.37 (1H, dd, $J = 8.8$, 2.4 Hz, H-5′), 7.89 (1H, d, $J = 8.8$ Hz, H-6′), 7.73 (1H, d, $J = 15.4$ Hz, H-β); $^{13}$C-NMR (CD$_3$OD) $\delta$: 127.9 (C-1), 131.8 (C-2), 116.9 (C-3), 161.5 (C-4), 116.9 (C-5), 131.8 (C-6), 114.7 (C-1′), 166.4 (C-2′), 103.9 (C-3′), 167.5 (C-4′), 109.2 (C-5′), 133.4 (C-6′), 118.4 (C-α), 145.7 (C-β), 193.6 (C=O). These data were identical to those in the literature [15,19].

(3R)-Vestitol (8). White crystals; $C_{16}H_{16}O_4$; $^1$H-NMR (CD$_3$OD), $\delta$: 3.93 (1H, t, $J = 10.1$ Hz, H-2a), 4.21 (1H, dd, $J = 10.1$, 4.1 Hz, H-2b), 3.42 (1H, m, H-3), 2.77 (1H, dd, $J = 15.5$, 4.1 Hz, H-4a), 2.93 (1H, dd, $J = 15.5$, 10.9 Hz, H-4b), 6.86 (1H, d, $J = 8.2$ Hz, H-5), 6.22 (1H, d, $J = 2.4$ Hz, H-8), 6.31 (1H, dd, $J = 8.2$, 2.4 Hz, H-3′), 6.37 (2H, m, H-6, 5′), 6.96 (1H, d, $J = 8.2$ Hz, H-6′), 3.71 (3H, s, 4′-OCH$_3$); $^{13}$C-NMR (CD$_3$OD), $\delta$: 71.2 (C-2), 33.2 (C-3), 31.4 (C-4), 131.2 (C-5), 109.1 (C-6), 156.4 (C-7), 103.9 (C-8), 157.3 (C-9), 115.0 (C-10), 121.5 (C-1′), 157.5 (C-2′), 102.5 (C-3′), 160.9 (C-4′), 105.8 (C-5′), 128.8 (C-6′), 55.6 (4′-OCH$_3$). These data were identical to those in the literature [19].

Sulfuretin (9). White crystals; $C_{15}H_{10}O_5$; $^1$H-NMR (CD$_3$OD), $\delta$: 6.85 (1H, d, $J = 8.2$ Hz, H-4), 7.24 (1H, d, $J = 8.2$ Hz, H-5), 6.70 (3H, overlapped, H-7, 10, 6′), 7.54 (1H, s, H-2′), 7.61 (1H, d, $J = 8.3$ Hz, H-5′); $^{13}$C-NMR (CD$_3$OD), $\delta$: 147.7 (C-2), 184.4 (C-3), 126.9 (C-4), 116.8 (C-5), 169.9 (C-6), 99.4 (C-7), 168.7 (C-8), 114.8 (C-9), 114.8 (C-10), 125.4 (C-1′), 114.3 (C-2′), 146.8 (C-3′), 149.7 (C-4′), 119.0 (C-5′), 126.5 (C-6′). These data were consistent with those previously reported [21].
3.4. Bacterial Strains

The *R. solanacearum* strain was obtained from Professor Ming-he Mo of the Key Laboratory of Protection and Utilization of Biological Resources, Yunnan University, and maintained on a nutrient agar (NA) slant at 4 °C.

3.5. Antibacterial Activity

These compounds were individually tested for *in vitro* antibacterial activity against *R. solanacearum* strain by the filter paper disc agar diffusion method [22]. The NA medium was mixed with suspension (2 mL) containing $10^7$ CFU/mL of *R. solanacearum*, and then poured into Petri-plates to a uniform depth of 5 mm and was allowed to solidify. The isolated compounds dissolved in dimethyl sulfoxide (DMSO) (1.6 µL, 50 mg/mL) were impregnated on sterile filter paper discs (6 mm diameter) and then applied aseptically to the surface of the agar plates. Streptomycin sulfate (1.6 µL, 50 mg/mL) was used as positive control. The plates were incubated at 37 °C for 24 h. Then the diameters of the inhibition zones including the 6 mm disc diameter were measured. Experiments were done in triplicate, and the results were mean values.

4. Conclusions

In conclusion, a total of nine compounds including four isoflavanones 1–4, two flavanones 5 and 6, one chalone 7, one isoflavane 8 and one aurone 9 were isolated from *D. odorifera* and identified by comparison of their NMR data with data reported in the literature. In addition, all compounds were evaluated for their inhibitory activity against *R. solanacearum*. Among the nine flavonoids, compound 8 exhibited the strongest antibacterial activity and compounds 2, 6, and 7 showed strong antibacterial activity. This is the first report of the anti-*R. solanacearum* activity of the compounds from *D. odorifera*.

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