· **Letters** ·

A New Compound with Anti-oxidative Activity from Seeds of *Jatropha curcas*

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Abstract: **Objective** To search for chemical constituents with anti-oxidative activity from seeds of *Jatropha curcas*. **Methods** DPPH radical scavenging assay was used to screen fractions or constituents with anti-oxidative activities. Active fractions were separated by varied chromatography and then identified on the basis of physiochemical properties and spectroscopic methods. **Results** The *n*-butanol layer of ethanol extract from the seeds of *J. curcas* showed stronger activity than other fractions and was studied further. A new compound was isolated from this active layer, and its structure was identified as jatrophasin A (3,4,4′,5′-tetrahydroxyl-3′-methoxyl-bisepoxylignan, **1**). It showed stronger anti-oxidative activity compared with resveratrol. **Conclusion** Compound **1** is a new compound which has never been reported with strong anti-oxidative activity.

Key words: anti-oxidative activity; DPPH; jatrophasin A; resveratrol; seeds of *Jatropha curcas*
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Introduction

Jatropha curcas L. is widely distributed throughout Southern China. Its whole plant can be used as Chinese herbal medicine; Its husk showed antibacterial activity and peptides, diterpenes, and triterpenes are main antibacterial compounds (Li *et al*, 2010); Its seeds have been usually reported to show activities, such as antitumor, insecticide and anti-oxidation (Openshaw, 2000). Isolation of curcin, curcain, and diterpenes proved the antitumor and insecticidal effects (Nath and Dutta, 1991). The aim of this work is to find active compounds from the seeds of *J. curcas*. Using activity-guided-isolation, a new compound was isolated, and its structure was elucidated by spectral and chemical methods.

Materials and methods

Equipments

^{1}H-NMR (CD_3OD) was taken on Bruker AV-400 MHz and ^{13}C-NMR was taken on Bruker AV-100 MHz, using TMS as internal standard. Mass spectra were recorded on Bruker Esquire 2000 ESI spectrometer. Optical rotations were measured with a Jasco P1020—OR. UV spectra were recorded on a Shimazu 2401PC. The IR spectra were measured with a Shimazu FTIR2400. HPLC chromatography was detected with Shimazu 10A-HPLC. Luna C_{18} analytic column (250 mm × 4.6 mm, 5 μm) from America Phenomenex Co., Shim-pack PREP-ODS prepared column (250 mm × 20 mm, 15 μm) from Japan Shimazu Co., Spectra max 340 pc Microplate Reader from America Molecular Device.

Plant material

The seeds of *Jatropha curcas* L. were collected from Yunnan Province, China, and identified by chief apothecary XIONG Ying (Shenzhen Institute for Drug Control, Shenzhen, China). A voucher specimen has been deposited in Shenzhen Research Center of Traditional Chinese Medicines and Natural Products, Shenzhen, China.

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Activity-guided-isolation

The dried seeds of *J. curcas* (4 kg) were peeled off and extracted by 95% ethanol for three times. The solvent was evaporated, suspended in water, and then extracted successively with benzine and *n*-BuOH. The DPPH (Lu, Hu, and Xu, 2003; Blois, 1958) activities of the three fractions have showed that activity of *n*-BuOH was stronger than others (When concentration was 25 mol/L, scavenging was 80.4%). So the *n*-BuOH soluble phase (47 g) was further investigated using silica gel and eluted by gradient $CHCl_3$ to give 15 fractions (Fr. 1—Fr. 15). Fr. 6 was isolated further to obtain compound **1** (1.2 mg).

Anti-oxidative activities (DPPH)

Samples and resveratrol (positive control) were dissolved by DMSO and diluted with ethanol to working concentrations. A mixture of ethanol and 0.4 mol/L HOAc-NaOAc (2 ∶ 1) buffer was prepared. DPPH was dissolved in ethanol to make 0.2 mg/mL solution. Mixture (120 μL), sample solution (40 μL), and DPPH (40 μL) were added into 96-well transparent plate in turn and incubated in dark for 30 min. The absorption at 517 nm was finally determined. DPPH scavenging activity of each sample was calculated according to the formula below:

$$\text{Scavenging rate} = \left(1 - \frac{A_S - A_B}{A_C - A_B}\right) \times 100\%$$

A_S is the absorption of samples; A_B is the basic absorption of the 96 orifice plates; A_C is the absorption of controls.

Results

Structural determination

Compound **1**: brown powder with $[\alpha]_D^{25}$ +6.1° (*c* = 0.10, MeOH). UV spectrum indicated the presence of carbon aromatic ring groups on the basis of these data as follows: 279.50 (3.44), 254.0 (3.40), 232.5 (3.96), 220.0 (4.09). Its molecular formula was determined to be $C_{19}H_{20}O_7$ on the basis of HR-ESI-MS (*m*/*z* 359.1103 (calculated for 359.1136) $[M - H]^-$). ^{1}H-NMR showed signals characteristic for one methoxy group at *δ* 3.83 (3H, s), aromatic signals at *δ* 6.79 (1H, d, *J* = 1.6 Hz), 6.73 (1H, d, *J* = 8.1 Hz), and 6.67 (1H, dd, *J* = 1.6, 8.1 Hz) indicated a 1,3,4-substituted benzene ring. ^{13}C-NMR of compound **1** showed 19 carbon signals for two aromatic rings at *δ* 102.7−149.8, one methoxy group at *δ* 56.7. In the COSY spectrum, the correlation between *δ* 3.07 and 4.62 and the correlation between 4.20 and 3.80 showed the presence of CH-CH-CH_2; the same both between *δ* 3.08 and 4.63 and between 4.22 and 3.79. In the HMBC spectrum, the correlation between *δ* 4.62 and both 55.3 and 72.7 implied the presence of a tetrahydrofuran ring; the same between *δ* 4.63 and both 55.5 and 72.6. The correlation between *δ* 6.67 and 87.4 indicated that the benzene ring with proton at 6.67 was connected to position 7, and the correlation between *δ* 6.50 and 87.6 indicated that the benzene ring with proton at 6.50 was connected to position 7′ of *bis*-tetrahydrofuran, respectively. Above all, we indicated the plane structure of compound **1** as shown in Fig. 1. In addition, it also supported by the unsaturation of this compound was 10. The coupling constant of *δ* 4.62 (H-7) and 3.07 (H-8) was 4.1 Hz, the same to *δ* 4.63 (H-7′) and 3.08 (H-8′), so we can infer the relative configuration is *trans* (Kamiya, 2004; Nakai, 2003). Compound **1** is a new compound which has never been reported yet. According to references (Kamiya, 2004), compound **1** was elucidated as jatrophasin A (3,4,4′,5′-tetrahydroxyl-3'-methoxyl-*bis*-epoxylignan). The NMR data of compound **1** can be found in Table 1.

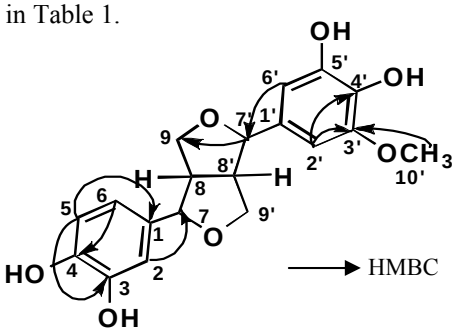

Fig. 1 HMBC correlation of compound 1

Radical scavenging activity

Using DPPH radical scavenging assay, three fractions of the extract and compound **1** were tested. From Fig. 2, we knew that the *n*-butanol layer of ethanol extract from the seeds of *J. curcas* showed stronger activity than any other fractions and was further studied. Compound **1** isolated from this active layer also showed stronger activity (IC_{50} = 22.85 μmol/L) compared to resveratrol (IC_{50} = 40.67 μmol/L), the positive control.

Discussion

There is little investigation about anti-oxidative

Table 1 NMR data of compound 1 (400 MHz, in CD_3OD)

Position	δ_C	δ_H
1	133.9	—
2	114.5	6.79 (1H, d, *J* = 1.6 Hz)
3	146.5	—
4	146.1	—
5	116.3	6.73 (1H, d, *J* = 8.1 Hz)
6	118.9	6.67 (1H, dd, *J* = 1.6, 8.1 Hz)
7	87.4	4.62 (1H, d, *J* = 4.1Hz)
8	55.5	3.07 (1H, br)
9	72.6	3.80 (1H, br), 4.20 (1H, br)
1′	133.2	—
2′	102.7	6.50 (1H, d, *J* = 1.8 Hz)
3′	149.8	—
4′	134.8	—
5′	146.7	—
6′	107.9	6.47 (1H, d, *J* = 1.8 Hz)
7′	87.6	4.63 (1H, d, *J* = 4.1Hz, H-7′)
8′	55.3	3.08 (1H, br)
9′	72.7	3.79 (1H, br), 4.22 (1H, br)
10′	56.7	3.83 (3H, s)

activities of *J. curcas* to be reported. In our research, we found the active fraction and some compounds including one new compound above. This study could provide guidance for further research about anti-oxidative activities from seeds of *J. curcas*.

Because condition was limited, we can't ensure the stereo-configuration. We expect to solve this problem in further research.

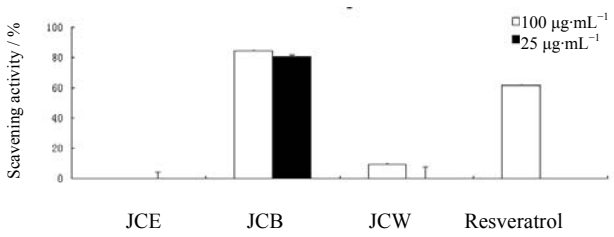

Fig. 2 Radical scavenging activities of fractions from seeds of *J. curcas*, with resveratrol as positive control

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