SHORT REPORT

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Antibacterial activities of crude extracts from physic nut (*Jatropha curcas*) seed residues

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ABSTRACT: In order to prepare four types of crude extracts, physic nut seed residues (seed cake) were extracted using a Soxhlet apparatus with organic solvents. Hexane, dichloromethane, acetone, and methanol were used to macerate the nut for 72 h to obtain crude extracts, namely, PH, PD, PA, and PM(H), respectively. In the case of cold extraction, physic nut seed residue was macerated with methanol for 72 h at room temperature resulting in the crude extract PM(C). Antibacterial activities of all crude extracts were determined against each of the Gram-negative bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*. and against the Gram-positive bacteria, *Staphylococcus aureus*, *Bacillus cereus*, and *B. megaterium*. This was done by using the agar diffusion method compared to ampicillin and streptomycin as positive control. The results showed that *E. coli* was inhibited by 30 mg/ml of PA and 50 mg/ml of PD. *P. aeruginosa* could not grow in the medium containing 50 mg/ml of PA. *S. aureus*, *B. cereus*, and *B. megaterium* were inhibited by PM(H) at different concentrations. PM(C) crude extract only inhibited *B. megaterium*. *Salmonella typhi* was not inhibited by any of the crude extracts.

INTRODUCTION

Jatropha curcas L. (physic nut, purging nut) is a medicinal plant used for the treatment of various diseases such as malarial fever, arthritis, gout, and jaundice. In some regions of Africa, physic nut leaves are used as a purgative [1,2]. Leaves of *J. curcas* contain flavonoids (apigenin, glycosides, vitexin, and isovitexin), sterols (stigmasterol, β-D-sitosterol, and sapogenins), alkaloids, and triterpenalcohol. The physic nut seed oil contains 12-deoxy-10-hydroxyphorbol and a polyunsaturated diterpene ester. Moreover, a protease (curcain) has been isolated and characterized from the latex of *J. curcas* [3]. In addition, a crude extract of *J. curcas* has been found to inhibit HIV-induced cytopathic effects with low cytotoxicity [4,5]. This paper describes the antibacterial activities of crude extracts from physic nut seed residue against some species of Gram-negative and Gram-positive bacteria.

MATERIALS AND METHODS

Plant materials

The physic nut seeds were collected from agricultural co-operatives of Phetchabun, Thailand in 2007. Physic nut seeds were cracked, the shells were removed and the kernels were obtained. Physic nut seed kernels were cut and sun-dried. The kernels obtained were used for oil extraction. The crude kernels are known as physic nut seed residue or seed cake.

Extraction

The *J. curcas* seed residue was cut and dried at room temperature. For PM(C) preparation, dried *J. curcas* seed residue (1200 g) was repeatedly extracted twice with methanol at room temperature for 72 h before filtration. The extracts were dried using a rotary evaporator at 50 °C. For other crude extracts, dried *J. curcas* seed residue (2300 g) was extracted using Soxhlet apparatus (EX5/75 BS2071, Quickfit, UK) with organic solvents for 72 h starting with hexane, dichloromethane, acetone, and methanol to obtain the crude extracts PH, PD, PA, and PM(H), respectively. The PD crude extracts were separated by quick column chromatography (sintered glass 250 ml, pore 4) with hexane, chloroform, acetone, and ethanol as mobile phases. Forty-nine fractions (200 ml/fraction) were obtained and the fraction PDD12 was percolated to afford Compound I (PDD). The PA crude extract (∼9 g) was further fractionated using vacuum column chromatography (sintered glass 250 ml,

pore 4). A step-wise gradient elution with hexane, chloroform, acetone and ethanol as mobile phases was used. Forty-five fractions (200 ml/fraction) were obtained and pooled to nine groups (PAG1–PAG9). PAG5 were separated on a Sephadex LH-20 column (2.5 cm × 150 cm) using acetone as eluent. Fourteen fractions (200 ml/fraction) were obtained and named PAD5/1–PAG5/14. Fraction PAG5/2 was recrystallized in hot methanol to yield Compound II (1.02 g, 11.22%). The extract samples and PDD were tested for their bacterial activities by using the agar diffusion method and were compared with the effect of the antibiotics ampicillin and streptomycin. Compound I and Compound II were analysed by spectroscopic techniques.

Antibacterial assay

Three species of Gram-negative bacteria, namely, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*, and three species of Gram-positive bacteria, *Staphylococcus aureus*, *Bacillus cereus*, and *B. megaterium*, were used to determine the minimum bacterial growth inhibition concentration of the crude extracts. After an aseptic swab, the assigned culture was put onto the appropriate plate. The bacteria were inoculated in three directions to ensure complete plate coverage. A disk was impregnated with the crude extract and placed on the surface of the agar. The disk was gently tapped with forceps to ensure better contact with the agar. This procedure was repeated three times, placing two disks of the same sample on the same plate. The plates were inverted (agar on top) during incubation at 37 °C for 18–24 h. The zone size was recorded using a ruler. The other bacteria and crude extracts were recorded with the same method.

RESULTS AND DISCUSSION

The quantities of crude extracts from hot and cold extraction were found to differ. For example, PM(H) 3.5% and PM(C) 2.7% (weight of dried seed residue) was obtained from hot and cold methanol extraction, respectively. The PH crude extract contained about 410 g (79.5% wt) of feature oil with a density of 0.91 g/ml and a boiling point of 270 °C. Therefore, PH extract was not tested for bacterial activities with the agar diffusion method. Other crude extracts, PD, PA, and PM(H) contained 13.1 g (2.5% wt), 11.7 g (2.3% wt), and 80.6 g (15.7% wt), respectively. Two antibiotic standards, ampicillin and streptomycin, showed an inhibition zone to *B. cereus* and *B. megaterium*, but the inhibition of the two antibiotic standards to *B. megaterium* was better than that to *B. cereus*. The PM(H) extract exhibited a better inhibition zone to *B. cereus* than to *B. megaterium*, and was better than the two antibiotic standards. Moreover, the crude extracts, PD, PA, and PM(C), were selectively inhibited to *B. megaterium*, even though PD, PA, and PM(C) extracts showed less activity than the two antibiotic standards. However, the concentration of 10 mg/ml of PDD showed an inhibition zone to *B. megaterium* which increased from PD at the same concentration (Table 1). In the case of Gram-negative bacteria, the concentration of 30 and 50 mg/ml PA showed inhibitory activity on two bacteria, *E. coli* and *P. aeruginosa*. Moreover, the concentration of 50 mg/ml PD was shown to be specifically selective on *E. coli*. However, all crude extracts and the two antibiotic standards showed negative activity to *S. aureus*. The results of inhibitory activities of crude extracts on three strains of Gram-negative bacteria are presented in Table 1.

Compound I (PDD) was a white solid with a melting point around 310 °C. The ^{13}C-NMR ($CDCl_3$) spectra showed more than 35 carbon atoms. The DEPT-135 technique showed methylene (–CH_2) peaks at δ 11.86, 11.98, 19.39, 22.68, 23.09, 24.70, 24.87, 25.63, 26.12, 27.16, 27.21, 28.24, 29.23, 31.52, 31.66, 31.92, 33.71, 37.27, 62.75, and 65.05 ppm, –CH/CH_3 peaks at δ 14.09, 19.39, 56.78, 129.73, 130.02, and 130.22 ppm, and quaternary carbon atoms at δ 68.41, 71.83, 121.72, 127.91, 128.07, 128.91, 130.54, 133.80, and 165.29 ppm. The IR (KBr) spectra confirmed that hydroxyl groups were not present in these structures. The spectra at a wavelength of 1722 cm^{-1} indicated a carbonyl group. The IR spectra also showed a medium-intensity peak in the range of 1300–1100 cm^{-1} which corresponds to the C−(C=O)−C of a ketone compound, consistent with C-13 data of the carbonyl carbon shown at δ 165.29 ppm.

Compound II was a pale yellow crystal with a melting point of 173 °C. The ^{13}C-NMR spectra exhibited 12 carbon atoms at δ 60.99, 62.55, 62.60, 70.34, 72.11, 73.28, 73.35, 74.78, 77.54, 83.03, 92.21, and 104.51 ppm. The DEPT-135 technique gave methylene (–CH_2) peaks at δ 60.99, 62.55, and 62.60 ppm, and –CH peaks at δ 70.34, 72.11, 73.28, 73.35, 74.78, 77.54, 83.03, and 92.21 ppm. The peak at δ 104.51 ppm cannot be observed by the DEPT-135 technique. This indicated that this carbon atom was a quaternary carbon. Moreover, the ^{1}H-NMR spectra showed signals for three methylene groups (δ 3.39, 3.41, 3.52, 3.55, 3.56, and 3.58 ppm) nine methane protons (δ 3.19, 3.21, 3.49, 3.57, 3.66, 3.78, 3.90, and 5.18 ppm), and seven hydroxyl protons (5.03–5.05, 4.81, 4.79, 4.78, 4.50–4.47, 4.41–4.40, and 4.39–4.37 ppm). The HMQC and HMBC spectra confirmed

Table 1 Inhibitory activity of crude extracts on three strains of Gram-positive bacteria and three strains of Gram-negative bacteria.

Crude extracts/ antibiotic	Concentration (mg/ml)	Inhibition zone ± SD (mm)				
		Gram-positive bacteria		Gram-negative bacteria		
		B. cereus	*B. megaterium*	*E. coli*	*P. aeruginosa*	*Salmonella typhi*
Ampicillin	10	2.7 ± 0.6	12.3 ± 0.6	18.3 ± 0.6	17.3 ± 0.6	15.3 ± 0.6
Streptomycin	10	0.5 ± 0.5	12.0 ± 0.0	13.0 ± 0.0	10.7 ± 0.6	10.3 ± 0.6
PD	10	0	3.4 ± 0.6	0	0	0
PA	10	0	2.7 ± 0.6	0	0	0
PM(H)	10	5.3 ± 0.6	2.0 ± 1.0	0	0	0
PM(C)	10	0	2.7 ± 1.5	0	0	0
PDD	10	0	6.0 ± 0.0	-	-	-
PD	30	0	4.3 ± 0.6	0	0	0
PA	30	0	3.3 ± 0.6	2.7 ± 0.6	4.3 ± 0.6	0
PM(H)	30	5.7 ± 1.2	2.3 ± 0.6	0	0	0
PM(C)	30	0	2.7 ± 2.1	0	0	0
PD	50	0	5.3 ± 0.6	2.3 ± 0.6	0	0
PA	50	0	4.0 ± 0.0	2.7 ± 1.2	6.3 ± 0.6	0
PM(H)	50	10 ± 1.0	5.0 ± 0.0	0	0	0
PM(C)	50	0	3.3 ± 2.5	0	0	0

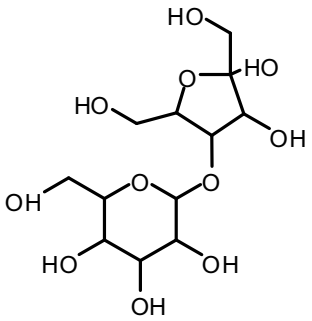

Fig. 1 Structure of lactulose.

the presence of three methylene, nine methane, and seven hydroxyl protons. IR (KBr) spectra confirmed the presence of a hydroxyl group at 3390 cm^{-1}, free –OH with a sharp band at 3563 cm^{-1}, sp^3 C–H absorption at 2940 cm^{-1}, $–CH_2$ characteristic bending absorption at 1439 cm^{-1}, and C–O stretching and C–O–C stretching absorptions in the range of 1279–1068 cm^{-1}. From spectroscopic data, compound II corresponds to lactulose with the structure shown in Fig. 1.

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