SUPPLEMENTARY MATERIAL

Assessing the biological activities of xanthone derivatives from *Swertia macrosperma* C.B. Clark

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The anti-microbial and anti-oxidant effects of xanthenes extract from *Swertia macrosperma* C.B. Clark were investigated using extracts from whole plant and partitioned by petroleum ether, ethyl acetate and n-butanol, respectively. Anti-microbial and anti-oxidant activities were detected among different fractions. High performance liquid chromatograph was performed to separate and purify the elutions. The compounds were elucidated by ¹H, ¹³C NMR and LCMS. The ethyl acetate extract showed maximum inhibitory activity in fungal organisms and Gram-positive bacteria, and had the highest anti-oxidant capacity. Eight xanthenes were isolated in ethyl acetate fraction from *Swertia macrosperma*. Moreover, compounds IV, V, VI and VIII were isolated from the plant for the first time, and compound VII had the strongest anti-oxidant effect.

Keywords: xanthone; *Swertia macrosperma*; isolation; petroleum ether; ethyl acetate; n-butanol

Experimental

Plant material

Plant material was collected in Tibet P.R. China in the middle of September 2013 and identified as whole plant of *Swertia macrosperma* C.B. Clark by Dr. Luo from Xining Institute for Food and Drug Control (xiong cheng wen-13-01). The plants were stored at room temperature in a cool, dry place.

Chemicals and apparatus

All the chemicals were chemical grade and purchased from Sinopharm Chemical Reagent Co., Ltd, China. The silica gel used in column chromatography and thin layer chromatography were purchased from Qingdao Haiyang Chemical CO., Ltd. Heparin sodium injection was purchased from Jiangsu Wanbang biochemical pharmaceutical Co., Ltd. The total anti-oxidant (T-AOC) capacity assay kit and malondialdehyde (MDA) kit were offered by Nanjing Jiancheng Biological Engineering Institute. Bovine serum albumin (BSA) V was purchased from Shanghai Jinsui Bio-Technology Co., Ltd.

A Shimadzu UV-2450 Spectrometer was performed to measure the ultraviolet spectra. A Shimadzu IR Prestige-21 FT-IR Spectrometer was used to detect the IR spectraby KBr method. A mettler FP5 was applied to calculate the melting points. The MS data and NMR data were measured with AB SCIEX QTEAP 5500 and Avance II 400 Bruker Biospin, respectively.

Experimental animals

A rabbit (male), weighed around 1.8 kg, was purchased from Lanzhou Institute of

Biological Products Co., Ltd, and was housed in a temperature and humidity controlled environment.

Extraction and isolation

The dried whole plant of *Swertia macrosperma* was treated into powder. The powder was extracted with 80% (v/v) ethanol aqueous solution under condition of reflux. All the extracts were combined and concentrated in reduced pressure and low temperature. Then, the crude extract was suspended in double-distilled water and treated with petroleum ether (PE), ethyl acetate (EA) and n-butanol (NB) in separator funnel, respectively. The EA fraction was analyzed on silica gel column with a gradient elution of petroleum ether-ethyl acetate and chloroform-methanol. The NB fraction was analyzed on polyamide column with a gradient elution of water-ethanol. The thin layer chromatography were performed to enrich the similar eluents. Finally, preparative high performance liquid chromatograph was performed to separate and purify the eluents. Isolated compounds were elucidated by ¹H and ¹³C NMR.

Disc diffusion assay

Disc diffusion assay was performed by the plates, 25 ml of sterilized nutrient agar (modified sabouraud Agar for *Candida albicans*) and 0.2 ml microbial suspension mixtures were gently poured into 90 mm plates, which ensured the thickness of plates were the same. Sterile circular glass tube was used to punch holes in the culture medium and the inner diameter was 6mm. The micro-organisms used were as followed: *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), *Escherichia coli* (*E. coli*), *Salmonella spp* (*S. spp*), and *Candida albicans* (*C. albicans*). The different fractions were dissolved at a final concentration of 20 mg/mL in dimethyl formamide. The volume for the different solutions was 50μl. Inhibition zone diameters with extracts or vancomycin (30μg) discs were measured at 37°C after 24h incubation. The inhibitory values were calculated from three replicates using vernier caliper.

Anti-oxidant activity

We investigated the plasma T-AOC capacity for the different fractions from isolation solutions and the isolated xanthones. Whole blood was isolated from the heart of a rabbit. Heparin, purchased from wanbang biopharmaceuticals, was used as anti-coagulant. The plasma was separated by centrifugation at 1000 g for 15 min at 4°C. The test solution was made of 0.1 mL plasma and 0.1 mL different fractions (dissolved in methanol at a concentration of 10 mg/mL). The T-AOC was determined according to the reference of kit after 30 min in 37°C water bath.

Moreover, we investigated the spontaneous lipid peroxidation of liver in rabbit for the different fractions from isolation solutions and the isolated xanthones by lipid peroxidation assay kit (Abcam, Cambridge, MA). The test solution was made of 0.9 mL liver tissue homogenate (10% saline suspension solution) and 0.1 mL different fractions (dissolved in methanol at a concentration of 10 mg/mL). The

malondialdehyde was determined by the reference of kit after 120 min in 37°C water bath. The inhibition rate (IR) was used to evaluate the anti-oxidant activity.

Table S1. ^{13}C NMR spectroscopic data for I-VIII in DMSO- d_6 (100MHz for ^{13}C).

Position	I	II	III	IV	V	VI	VII	VIII
1	163.4	162.5	162.4	162.4	162.7	163.2	162.7	162.5
2	97.3	98.3	98.0	97.6	98.7	97.8	98.9	97.9
3	166.7	167.7	167.6	167.5	167.0	166.5	167.0	167.6
4	92.4	93.5	93.5	93.2	94.5	92.6	94.8	93.5
4a	157.2	157.9	157.9	158.2	158.3	156.7	157.9	157.9
4b	150.5	145.1	143.9	148.5	147.5	147.1	152.3	143.9
5	113.2	109.6	137.8	106.5	140.9	125.0	107.8	137.8
6	121.6	121.4	124.4	124.7	124.4	121.9	124.2	124.4
7	148.2	153.3	110.0	141.0	107.7	118.6	137.7	110.0
8	149.6	140.1	152.3	147.6	148.4	153.4	143.8	152.3
8a	115.4	108.1	108.0	107.9	106.4	106.2	109.9	108.0
8b	103.7	102.8	102.7	102.2	101.3	104.0	101.7	102.6
9	181.0	184.3	184.6	184.7	184.4	180.9	184.2	56.7
3-OCH ₃	56.5	56.8	56.8	56.7		56.6		
5-OCH ₃						56.8		
7-OCH ₃	57.2							
8-OCH ₃	61.5	58.1				57.0		

Table S2. ¹H NMR spectroscopic data for I-VIII in DMSO-d₆ (400MHz for ¹H)

Position	I	II	III	IV	V	VI	VII	VIII
1	13.205(1H,s,-OH))	11.815(1H,s,-OH)	11.925(1H,s,-OH)	11.866(1H,s,-OH)	11.879(1H,s,-OH)	13.269(1H,s,-OH)		
2	6.324(1H,s)	6.415(1H,s)	6.426(1H,s)	6.366(1H,s)	6.195(1H,d,J=2.0Hz)	6.336(1H,d,J=1.6Hz)	6.222(1H,s)	6.408(1H,s)
3					9.383(1H,s,-OH)			
4	6.509(1H,s)	6.682(1H,s)	6.638(1H,s)	6.572(1H,s)	6.337(1H,d,J=1.6Hz)	6.582(1H,s)	6.413(1H,s)	6.620(1H,s)
5	7.326(1H,d,J=8.4Hz)	6.733(1H,d,J=8.8Hz)	9.748(1H,s,-OH)	6.888(1H,d,J=8.8Hz)	6.888(1H,d,J=8.8Hz)		9..629(1H,s,-OH)	

6	7.604(1H,d,J=8.4Hz)	7.467(1H,d,J=8.8Hz)	7.259(1H,d,J=8.8Hz)	7.289(1H,s)	7.274(1H,s,J=9.2Hz)	7.473(1H,d,J=9.2Hz)	7.253(1H,d,J=9.2Hz)	7.279(1H,d,J=8.8Hz)
7		11.241(1H,s,-OH)	6.671(1H,d,J=8.8Hz)	9.376(1H,s,-OH)		6.918(1H,d,J=9.2Hz)	6.640(1H,d,J=9.2Hz)	6.662(1H,d,J=8.8Hz)
8			11.092(1H,s,-OH)	11.616(1H,s,-OH)	11.716(1H,s,-OH)			11.160(1H,s,-OH)
3-OC H3	3.824(3H,s)	3.886(3H,s)	3.907(3H,s)	3.879(3H,s)		3.902(3H,s)		
5-OC H3						3.874(3H,s)		3.904(3H,s)
7-OC H3	3.869(s)							
8-OC H3	3.869(s)	3.900(3H,s)				3.846(3H,s)		

Table S3. Anti-microbial and anti-fungal activity of PE, EA and NB fractions of *Swertia macrosperma* (Mean $\pm$ SEM, mm).

	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>S. spp</i>	<i>C. albicans</i>
PE fraction	9.8 $\pm$ 0.5	12.0 $\pm$ 0.3	11.2 $\pm$ 0.6	10.9 $\pm$ 0.4	10.0 $\pm$ 0.4
EA fraction	13.1 $\pm$ 0.2	12.8 $\pm$ 0.4	12.1 $\pm$ 0.4	11.9 $\pm$ 0.4	13.1 $\pm$ 0.3
NB fraction	0	7.9 $\pm$ 0.2	14.8 $\pm$ 0.4	13.1 $\pm$ 0.3	9.0 $\pm$ 0.3
vancomycin	23.2 $\pm$ 0.3	22.1 $\pm$ 0.4	20.6 $\pm$ 0.2	19.8 $\pm$ 0.3	19.1 $\pm$ 0.3

Table S4. T-AOC and anti-lipid peroxidation of different fractions for *Swertia macrosperma*.

	Concentration (mg/mL)	T-AOC (U/mL)	IR (%)
Control	—	7.8	—
PE fraction	10	74.6	57.5
EA fraction	10	163.8	68.6
NB fraction	10	50.2	45.7

Table S5 The relationship between T-AOC, IR and different concentrations of EA fraction.

Concentration (mg/mL)	T-AOC (U/mL)	IR (%)
2	89.3	38.6
4	134.2	41.5
6	148.5	46.7
8	150.1	53.2
10	163.8	68.6

Table S6 T-AOC and anti-lipid peroxidation of eight xanthones isolated from *Swertia macrosperma*

	T-AOC(U/mL)	IR(%)
Control	8.1	—
I	90.6	6.92
II	112.7	3.31
III	148.0	16.9
IV	158.9	42.54
V	181.4	40.6
VI	78.1	9.57
VII	192.8	46.78
VIII	170.2	37.9

Figure S1. ¹³C and ¹H NMR spectra of compound I.

Figure S3. ¹³C and ¹H NMR spectra of compound III.

Figure S4. ¹³C and ¹H NMR spectra of compound IV.

Figure S5. ^{13}C and ^1H NMR spectra of compound V.

Figure S6. ¹³C and ¹H NMR spectra of compound VI.

Figure S7. ¹³C and ¹H NMR spectra of compound VII.

Figure S8. ¹³C and ¹H NMR spectra of compound VIII.