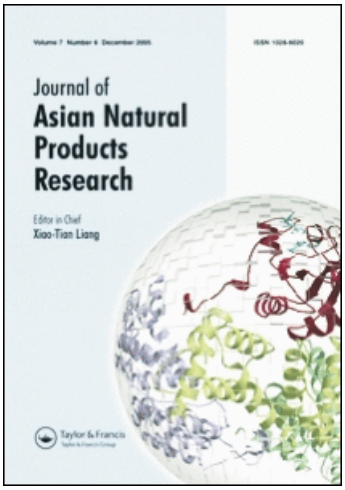

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EVALUATION OF TOXICITY OF SOME SAPONINS ON BRINE SHRIMP

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Toxicity of several types of saponins (**1–11**) against brine shrimp (*Artemia salina*) were evaluated. As a result, it was found that most tested compounds were not toxic to brine shrimp at high enough concentration. The most toxic saponin (**1**) to brine shrimp showed also cytotoxicity towards HL-60 tumor cell line using MTT assay. Brine shrimp model may thus be used as bench-top assay in finding cytotoxic components from saponin-containing fractions of plant extracts.

Keywords: *Artemia salina*; Brine shrimp test; Bioassay methods; Saponin; Cytotoxicity

INTRODUCTION

The brine shrimp (*Artemia salina*) lethality test has been used as a simple cytotoxic bioassay for natural product research [1]. Several types of cytotoxic compounds have been found by using this method, such as sesquiterpenes [2], diterpenes [3], alkaloids [4], saponins [5] and annonaceous acetogenins [6]. Among above compounds, the most impressive work came from McLaughlin's group during their investigations on acetogenins from the family Annonaceae [6]. It is well known that many saponins possess haemolytic and spermicidal activities as well as fish poisoning function due to their glycosidic structures. Whether these saponins can also kill the aquatic animal *Artemia salina* at low concentration and whether this kind of lethality is

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parallel to cytotoxicity against tumour cell line are critical to the adoption of brine shrimp model as general cytotoxic bioassay to plant extracts with high polarity. In this paper, we report our test results of toxicity of several types of saponins on brine shrimp and the finding of a cytotoxic saponin against HL-60 tumor cell line by using this method.

RESULTS AND DISCUSSION

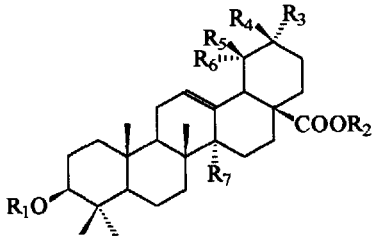
Toxicity of eleven saponins (**1**–**11**, Fig. 1) isolated previously in our laboratory, including both tetracyclic and pentacyclic as well as both monodesmosidic and bisdesmosidic triterpenoid glycosides were tested on brine shrimp, which gave us a more accurate evaluation of the action of saponins against the aquatic animal *Artemia salina*. Among those saponins tested, **1**–**3** were isolated from *Alternanthera philoxeroides* [7], **4**–**8** were from *Mussaenda pubescens* [8], and **9**–**11** were from *Panax ginseng*. The LC_{50} values of saponins **1**–**11** to brine shrimp were listed in Table I. Saponin **1**, the only compound with LC_{50} value below 100 μg/ml was further subjected to anti-cancer screening *in vitro* on HL-60 cell line using MTT assay. As a result, **1** inhibited the growth of HL-60 cell line at 10% (1 μg/ml), 29% (10 μg/ml) and 100% (100 μg/ml), respectively.

The above results showed that most saponins were not toxic to brine shrimp even at 1000 ppm, a much higher concentration than generally needed for cytotoxic natural products by using this assay. Brine shrimp model may thus be used as a cytotoxic assay to saponin-containing plant extracts, and also to pure saponins as it is used to other types of natural products.

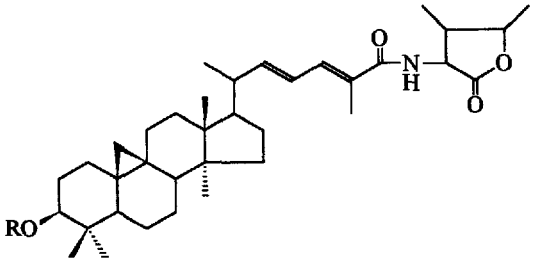
EXPERIMENTAL SECTION

Experimental Section Saponins tested in this work were purified previously in our laboratory. The eggs of *Artemia salina* was purchased from Malaysia (C.S.L. THEAN YEANG AQUARIUM SDN. BHD.) and the sea salt used for hatching *Artemia salina* was made in France (Instant Ocean aquarium systems). MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) and dimethyl sulfoxide were from Sigma Chemical Co.

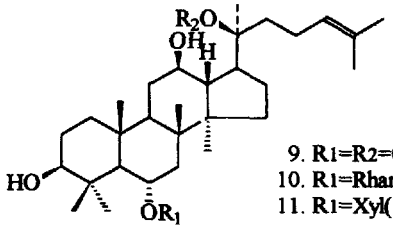
Shrimp hatching and incubation The eggs of *Artemia salina* were hatched in 3.5% artificial sea water. After incubation at 25–30°C for 48 h at least 20 nauplii were transferred into test tubes with 3.5% salty water soultions of



1. R1=GluA, R 2=R5=R6=H, R3=R4=R7=CH3
2. R1=GluA, R 2=Glu, R 3+R4=CH2, R5=R6=H, R7=CH3
3. R1=GluA, R 2=Glu, R 3=R4=R7=CH3, R5=R6=H
4. R1=R2=Glu, R 3=R5=R7=CH3, R4=H, R6=OH
5. R1=R2=Glu, R 3=R4=CH3, R5=R6=H, R7=COOH



6. R=[Rham(1-2)Glu(1-2)]Rham(1-4)Glu
7. R={[Glu(1-6)]Rham(1-2)Glu(1-2)}Rham(1-4)Glu
8. R={[Glu(1-2)Glu(1-6)]Rham(1-2)Glu(1-2)}Rham(1-4)Glu



9. R1=R2=Glu
10. R1=Rham(1-2)Glu, R 2=Glu
11. R1=Xyl(1-2)Glu, R 2=Glu

FIGURE 1 Structures of saponians 1–11.

saponins **1–11** at a final concentration of saponins at 1000, 200, 100, 50 or 10 ppm, respectively. All the tubes were incubated at 25–30°C for another 24 h.

Determination of LC_{50} LC_{50} values of saponins were determined using Finney Program provided by Prof. J.L. McLaughlin, Purdue University [9].

TABLE I Brine shrimp bioassay results for saponins **1–11**

Saponin	*Percent deaths at* 24 h					LC_{50} μg/ml	95% *Confidence limit*
	10 μg/ml	50 μg/ml	100 μg/ml	200 μg/ml	1000 μg/ml		
Control	0	0	0	0	0	—	—
1	0	20	60	87	88	98.5	69.9–136.7
2	—	—	—	0	10	>1000	
3	—	—	—	0	10	>1000	—
4	—	—	—	5	30	>1000	
5	—	—	—	10	25	>1000	
6	—	—	—	5	10	>1000	
7	—	—	—	15	20	>1000	
8	—	—	—	5	5	>1000	
9	—	—	—	0	0	>1000	
10	—	—	—	0	0	>1000	
11	—	—	—	0	0	>1000	

Cell culture The HL-60 cell line was obtained from the American Type Culture Collection. Cells were cultured in RPMI-1640 (Gibco BRL) medium containing 100 U/ml penicillin and 100 μg/ml streptomycin supplemented with 10% bovine serum at 37°C in humidified air containing 5% CO_2. In all experiments, exponentially growing cells were used.

MTT assay MTT assay was used to assess the cytotoxicity of saponin **1**. Single cell suspension of HL-60 was prepared, and the cell density was measured. An equal number of cells were inoculated into each well in 0.18 ml of culture medium to which 0.02 ml of 10 times the final concentration of drug or PBS (for untreated 100% survival control) was added. After 72 h, 0.1 mg of MTT was added to each well and incubated at 37°C for 4 more hours. Plates were centrifuged at 450 *g* for 5 min at room temperature and the medium was then aspirated, taking care not to disturb the formazan crystals at the bottom of the wells. Dimethyl sulfoxide (150 μl) was added to each well to solubilize the crystals. The plates were read immediately at 540 nm on a spectrophotometer. All experiments were performed three times.

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