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Short communication

In vitro anti-tumor promoting and anti-parasitic activities of the quassinoids from *Eurycoma longifolia*, a medicinal plant in Southeast Asia

Suratwadee Jiwajinda [a,*], Vilai Santisopasri [b], Akira Murakami [c], Masanori Kawanaka [d], Hiromu Kawanaka [d], Monique Gasquet [e], Riad Eilas [e], Guy Balansard [e], Hajime Ohigashi [f]

[a] *Central Laboratory and Greenhouse Complex, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand*
[b] *Department of Biochemistry, Faculty of Science, Kasetsart University, Chatuchak, Bangkok 10900, Thailand*
[c] *Department of Biotechnological Science, Faculty of Biology-Oriented Science and Technology, Kinki University, Iwade-Uchita, Wakayama 649-6493, Japan*
[d] *National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo 162, Japan*
[e] *Department of Pharmacognosy and Parasitology, Faculty of Pharmacy, University of Marseille II, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 5, France*
[f] *Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan*

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Abstract

Some quassinoids (**1**–**6**) isolated previously as plant growth inhibitors from the leaves of *Eurycoma longifolia* Jack. (Simaroubaceae) were subjected to in vitro tests on anti-tumor promoting, antischistosomal and plasmodicidal activities. The most active compound for inhibition of tumor promoter-induced Epstein–Barr virus activation (anti-tumor promotion) was 14,15β-dihydroxyklaineanone (**5**, $IC_{50} = 5$ μM). Longilactone (**1**) gave significant antischistosomal effect at a concentration of 200 μg/ml. 11-Dehydroklaineanone (**3**) and 15β-*O*-acetyl-14-hydroxyklaineanone (**6**) showed potent plasmodicidal activity ($IC_{50} = 2$ μg/ml). Thus it was suggested that *E. longifolia* possesses high medicinal values due to the occurrence of a variety of quassinoids. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Quassinoid; *Eurycoma longifolia*; Simaroubaceae; Anti-tumor promoting activity; Anti-parasitic activities

1. Introduction

Eurycoma longifolia Jack., a plant in the family Simaroubaceae, is one of the most well known folk medicines for antipyretic, antimalarial and restorative activities in Southeast Asia (Perry, 1980), and is known to be a promising natural source of biologically active compounds (Okano et al., 1990). Some of the constituents have been known to possess antiamoebic (Le and Nguyen, 1970), cytotoxic, antitumoral (Itokawa, et al., 1993) and plasmodicidal activities (Chan, et al., 1986). In particular, the quassinoids are one of the major bioactive groups in this plant. Previously, we have isolated six quassinoids (Fig. 1) from *E. longifolia* during the course of the search for plant growth inhibitors occurring in the medicinal plants of Thailand (Jiwajinda et al., 2001). In this short communication, in vitro anti-tumor promoting, antischistosomal and plasmodicidal activities of these quassinoids are reported.

2. Materials and methods

2.1. Plant material

Leaves of *E. longifolia* Jack. were collected at Surajthani, Thailand. A voucher specimen (specimen

* Corresponding author. Tel.: +66-34-351-399; fax: +66-34-351-392
E-mail address: rdiswj@nontri.ku.ac.th (S. Jiwajinda).

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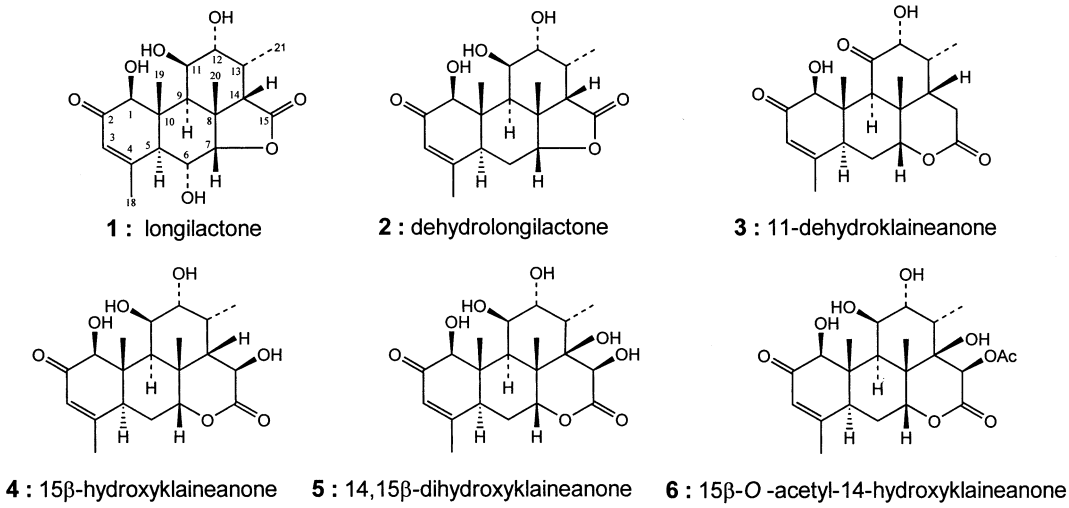

Fig. 1. Structures of the quassinoids isolated from *E. longifolia* (**1**–**6**).

no. BK 4316, Collector: PUT no. 2724) is kept in Bangkok Herbarium, Department of Agriculture, Ministry of Agriculture and Co-operation, Bangkok, Thailand.

2.2. Extraction and isolation

Quassinoids (**1**–**6**) were extracted and isolated from the leaves of *E. longifolia* as reported previously (Jiwajinda et al., 2001).

2.3. Bioassay

2.3.1. Inhibitory assay of Epstein–Barr virus (EBV) activation

In vitro anti-tumor promoting activity evaluated by the inhibition of tumor promoter induced EBV activation was carried out according to Murakami et al. (1998). Briefly, human B-lymphoblastoid cells, Raji, were incubated in 1 ml of RPMI 1640 medium (supplemented with 10% fetal bovine serum) containing sodium *n*-butyrate (440 μg), 12-*O*-hexadecanoylphorbol-13-acetate (HPA) (40 ng) and the test compound at 37 °C under 5% CO_2 atmosphere for 48 h. EBV activation was measured by detection of early antigen (EA), stained by a conventional indirect immunofluorescence method with high-titer EA-positive sera from NPC patients followed by FITC labeled IgG. The ratio of EA-induced cells was compared to that of a control experiment using only sodium *n*-butyrate and HPA, in which the ratio of EA-induced cells was ordinarily around 50%.

2.3.2. Antischistosomal activity test

In vitro antischistosomal activity was assayed using the method reported previously by Ohigashi et al. (1994). Briefly, an adult pair of schistosomes of *Schistosoma japonicum* was cultured in RPMI 1640 supplemented with 10% fetal calf serum (1 ml). After incubation for 24 h, inhibition of both the movement and egg-laying capability of the schistosomes in triplicate experiments were evaluated by three ranks (++, + and −) and the average number of eggs laid was determined as indicated in the previous report. The complete inhibition (++) and incomplete inhibition (+) activities were found to be irreversible, when schistosomes were treated with test compound for 24 h. No complete inhibition was detected in this study. Commercially available praziquantel was used as a positive control.

2.3.3. Plasmodicidal activity test

In vitro plasmodicidal activity was assayed using the method reported by Rathelot et al. (1995). Briefly, an ACC Niger chloroquine resistant *Plasmodium falciparum* strain was used. The parasite was cultured on glucose-enriched RPMI 1640 medium supplemented with HEPES and 10% human serum. The test procedure was followed by the method of Trager and Polonsky (1981). Each concentration was tested in triplicate. Giemsa-stained thin blood smears were examined under 1000 x magnification, and the percentage of parasited red blood cells were counted on at least 9000 red blood cells for each concentration. Percentage growth inhibition of the parasite was determined as indicated previously (Rathelot et al., 1995).

3. Results and discussion

In anti-tumor promoting activities tested by the inhibition of tumor promoter-induced Epstein–Barr

Table 1
Inhibitory effect of the quassinoids against tumor promoter-induced EBV activation

Compound	Concentration (μM)	Inhibition (%)	Cell viability (%)	IC_{50} (μM)
1	50	100.0	98.4	
	10	36.3	92.6	20.0
	1	16.1	94.6	
2	50	100.0	92.7	
	10	57.7	93.1	9.0
	1	0.0	98.0	
3	50	100.0	89.5	
	10	26.4	96.0	28.0
	1	12.8	97.2	
4	50	100.0	87.3	
	10	20.2	90.5	32.0
	1	1.6	90.3	
5	50	100.0	100.0	
	10	85.3	97.7	5.0
	1	13.2	98.8	
6	50	48.9	95.6	
	10	9.1	94.5	51.0
	1	1.9	93.8	
β-Carotene				30.0
Quercetin				23.0

virus (EBV) activation (Murakami et al., 1998), most of the isolated quassinoids showed complete inhibition at a concentration of 50 μM, except compound **6**. The most potent inhibitor was 14,15β-dihydroxyklaineanone (**5**) whose IC_{50} was 5.0 μM (Table 1). This inhibitory potential was much higher than that of quercetin ($IC_{50} = 23$ μM) and β-carotene ($IC_{50} = 30$ μM), two common anti-tumor promoting natural agents (Murakami et al., 1998). Compound **2** also showed significant activity ($IC_{50} = 9.0$ μM), and **1**, **3** and **4** were comparable inhibitors to querecetin or β-carotene, while **6** was classified as a less active compound. While the activities of compound **2** and **5** are thought to be comparable with that of brucine-D, which has also been isolated from *Brucea javanica* (Okano et al., 1995), they might be even stronger than those of nigakilactone-L isolated from *Picrasma ailanthoids* (Okano et al., 1995) and ailantinol C from *Ailanthus altissima* (Kubota et al., 1997). Based on previous reports, bruceanol E, a C_{20}-type quassinoid from *Brucea antidysenterica*, was supposed to be the most potent inhibitor ($IC_{50} < 1$ μM) of EBV activation (Okano et al., 1995) among the quassinoids so far

Table 2
Antischistosomal and plasmodicidal activities of the quassinoids

Compound	Antischistosomal activity[a]						Plasmodicidal activity[e]
	200 μg/ml		20 μg/ml		2 μg/ml		IC_{50} (μM)
	IM[b]	EL[c]	IM[b]	EL[c]	IM[b]	EL[c]	
1	+	20±16	+	133±108	−	933±177	5.5–13.7
3	+	193±34	+	306±306	−	853±537	5.3
4	NT	NT	NT	NT	NT	NT	5.3
5	+	113±146	+	166±165	−	833±99	5.0
6	NT	NT	NT	NT	NT	NT[f]	23.8
Control (DMSO-1) [d]	−	1273±328					
Control (DMSO-2) [d]	−	726±310					
Praziquantel (2 μg/ml)	+	0					
Chloroquine diphosphate							0.39

[a] Tested using *S. japonicum* in triplicate experiments.
[b] IM, inhibition of movement of adult schistosomes; +, incomplete inhibition; −, no inhibition.
[c] EL, number of eggs laid.
[d] Data in the medium containing 1% DMSO (v/v) in triplicate experiments.
[e] Tested using *P. falciparum* W2 (chloroquine resistant strain)
[f] NT, not tested.

isolated. Compounds **2** and **5** may be concluded to be the next potent of the quassinoids in inhibition of EBV activation.

Antischistosomal (Ohigashi et al., 1994) and plasmodicidal (Rathelot et al., 1995) activities were tested on only a part of the quassinoids because of sample limitation. Compounds **1**, **3** and **5** showed significant inhibitory effects on adult schistosome movement (IM) and egg-laying (EL) of *S. japonicum* at 200 μg/ml as compared with those of control experiments using only DMSO (Table 2). At a concentration of 20 μg/ml, all compounds inhibited movement of schistosomes and had slight effects on egg-laying. However, the antischistosomal effects of three quassinoids were evaluated to be weaker than that of a known drug, praziquantel (Table 2). In plasmodicidal tests using *P. falciparum* W2 (chloroquine resistant strain), **1** showed activities at the IC_{50} between 5.5 and 13.7 μM, **3**, **4**, **5** and **6** at IC_{50}s of 5.5, 5.3, 5.0 and 23.8 μM, respectively (Table 2). However, these IC_{50} values were higher than that of chloroquine diphosphate ($IC_{50} = 0.39$ μM), eurycomanone ($IC_{50} = 0.27$ μM) and eurycomanol ($IC_{50} = 0.68$ μM), all of which are C_{20}-type quassinoids with a $-CH_2O-$ bridge between C_8 and C_{11} found in the same plant, *E. longifolia* (Chan et al., 1986). Thus, this bridge may be one of the important part structures for anti-plasmodicidal activity of the quassinoids. Though the quassinoids tested in the present study showed only medium anti-parasitic activities, they may contribute to choosing further important part structures for the activity. Additional structure–activity studies of the quassinoids are needed.

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