BIOLOGICAL ACTIVITIES OF CRUDE EXTRACTS AND ZEYLENOL FROM *Uvaria grandiflora*

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Abstract: Chemical constituents of crude ethyl acetate extracts from the stem of *Uvaria grandiflora* resulted in the isolation and structural characterization of a zeylenol. The structure elucidation was carried out by 1D and 2D NMR techniques including ^{1}H and ^{13}C NMR, COSY, HMQC and HMBC, as well as other spectroscopic methods such as UV, IR and MS. The bioactivity assays revealed that crude ethyl acetate and acetone extracts showed antibacterial activity and zeylenol showed both antibacterial and antiinflammatory activities. The crude ethyl acetate extract was effective against 2 strains, *Escherichia coli* (EPEC) DMAT30546 and *Vibrio cholera* non 01 and the crude acetone extract was effective against 4 strains, *Staphylococcus aureus* ATCC25923, *Salmonella typhi* DMST22842, *Staphylococcus typhinurium* ATCC13311 and *Enterobacter cloacae* ATCC23355. Moreover, the zeylenol was effective against 4 strains of *E. coli* (EPEC) DMAT30546, *E. coli* O157:H7 DMST12743, *S. typhi* DMST22842 and *S. aureus* ATCC25923 with the same MIC values of 1,000 µg/mL. Antiinflammatory activity, zeylenol at the dose of 1 mg/ear produced significant inhibitory activity on the edema formation of 89.58, 68.52, 51.64 and 51.72 % at 15, 30, 60 and 120 min, respectively.

Introduction: The genus *Uvaria* is one of the largest paleotropical genera in the family Annonaceae, comprising more than 220 species distributed in wet tropical regions of Africa, Madagascar, South-East Asia, northern Australia and Melanesia. *Uvaria grandiflora* was found in the South and Southeast regions of Thailand. Plants in this genus have been studied for bioactive constituents and various classes of compounds such as alkaloids, annonaceous acetogenins, flavonoids were isolated. These compounds showed antimalarial, antitumor, pesticidal and other biological activities. Moreover, phytochemical investigation of *U. grandiflora* demonstrated the presence of several groups of natural chemical constituents; including polyoxygenated cyclohexenes and aromatic derivatives which showed interesting antitumor and antimalarial activities.[1-4] Polyoxygenated cyclohexenes have the core molecular skeleton of 1-methylcyclohex-4-ene. However, they contain multiple oxygenic substituents, such as benzoxyl, hydroxyl, alkoxyl, epoxyl and acetoxyl groups, and have varied stereochemistries.[5] In the present study, we report the isolation and characterization of polyoxygenated cyclohexene derivative, zeylenol (**1**) (Fig. 1) from crude ethyl acetate of *U. grandiflora* as well as its bioactivities.

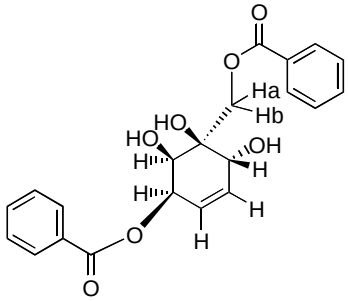

Figure 1. Structure of zeylenol

Methodology:

Phytochemical investigation

Air-dried powdered stems of *U. grandiflora* (2.2 kg) were extracted successively at room temperature with hexane, ethyl acetate and acetone (15 L for each solvent) to afford crude hexane (34.61 g), ethyl acetate (56.63 g) and acetone (54.02 g) extracts, respectively. Further separation of crude ethyl acetate extract (56.00 g) by silica gel column chromatography was carried out using eluents with increasing polarity of a gradient of hexane and ethyl acetate to afford eight subfractions. Subfraction A_6 was rechromatographed by the same procedure to afford a white solid which was recrystallized from 95% ethanol to obtain compound **1** (1.12 g).

Antiinflammatory assay

Ethyl phenylpropiolate (EPP)-induced ear edema in rats: The method of Brattsand et al. was used.[6] Male rats weighing 40-60 g were used. Ear edema was induced by the topical application of either EPP dissolved in acetone to the inner and outer surfaces of both ears by means of an automatic microliter pipet. Zeylenol, at the dose of 1 mg/ear, was dissolved in acetone and applied topically in a volume of 20 μl to the inner and outer surfaces of the ear just before the irritants. The control group was treated with acetone.

Antibacterial assay

Zeylenol was assessed for antibacterial activity against 9 bacteria strains (all obtained from The National Institute of Health, Department of Medical Sciences, Bangkok) by minimum inhibitory concentration (MIC) value. Determination of the MIC was carried out according to the modified resazurin assay described by Sarker et al. with some modifications.[7]

Results, Discussion and Conclusion: Crude extracts from stems of *U. grandiflora* were tested for antibacterial activity. The results demonstrated that the crude hexane extract showed inactive against all strains which cause diarrhea, the crude ethyl acetate extract was effective against 2 strains and the crude acetone extract was effective against 4 strains. (Table 1)

Table 1. Antibacterial activity of crude extracts from stems of *U. grandiflora*

Bacteria strain	Clear zone (mm)			
	Crude extract			Genta
	Hexane	EtOAc	Acetone	(Positive control)
E. coli (EPEC)	-	18.00	-	21.75
V. cholera	-	9.50	-	12.75
S. aureus	-	-	12.25	14.50
S. typhi	-	-	8.75	14.00
S. typhinurium	-	-	9.25	24.75
E. cloacae	-	-	12.50	21.50

Zeylenol was obtained from the crude ethyl acetate extract of stems of *U. grandiflora*. This compound was effective against 4 strains including *E. coli* (EPEC) DMAT30546, *E. coli 0157:H7* DMST12743, *S. typhi* DMST22842 and *S. aureus* ATCC25923 with the same MIC values of 1,000 µg/mL. (Table 2)

Table 2. Antibacterial activity (MIC, MBC) of zeylenol from *U. grandiflora* and gentamicin against various strains of bacteria (µg/mL)

Bacteria strain	zeylenol			Gentamicin		
	MIC	MBC	MIC/MBC	MIC	MBC	MIC/MBC
E. coli ETEC	NA	NA	-	0.3125	-	-
E. coli EPEC	1000	>1000	-	3.125	2.5	1.25
E. coli O157:H7	1000	>1000	-	0.625	0.625	1
S. typhi	1000	>1000	-	0.3125	0.625	0.5
S. typhinurium	NA	NA	-	0.3125	-	-
S .aureus	1000	>1000	-	0.15	1.25	0.12
E. aerogenes	NA	NA	-	0.625	-	-
E. choucea	NA	NA	-	0.625	-	-
P. milabilis	NA	NA	-	1.25	-	-

Zeylenol probably possesses antiinflammatory activity by inhibition of the release or synthesis of various inflammatory mediators (Table 3). The test zeylenol produced significant inhibitory activity at the dose of 1 mg/ear on edema formation at all determination times, with similar intensity as phenylbutazone. This compound at the dose of 1 mg/ear produced significant inhibitory activity on the edema formation of 89.58, 68.52, 51.64 and 51.72 % at 15, 30, 60 and 120 min, respectively. The antiinflammatory activity of zeylenol demonstrated that this compound showed similar intensity of activity as that observed for phenylbutazone.

Table 3. Antiinflammatory activity of zeylenol from *U. grandiflora*

Group	Dose (mg/ear)	Edema thickness (µm)				% edema inhibition			
		15 min	30 min	1 h	2 h	15 min	30 min	1 h	2 h
Control (Acetone)	-	160.00 ±7.30	180.00 ±5.16	203.33 ±8.03	193.33 ±4.94	-	-	-	-
Phenylbutazone	1	30.00± 13.41*	43.33± 9.54*	73.33± 12.29*	90±4.4 7*	81.25	75.93	63.93	53.45
zeylenol	1	16.67± 3.33*	57.67± 2.11*	98.33± 8.33*	93.33± 5.58*	89.58	68.52	51.64	51.72

Results are expressed as mean ± S.E.M. (N of ears = 6)
Significantly different from the control group: * $p < 0.05$

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Keywords: antibacterial activity, antiinflammatory activity, zeylenol, Annonaceae, *Uvaria grandiflora*