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Short Communication: Bioactive Materials

Anticancer and Antimicrobial Activities of 13(*E*)-labd-13-ene-8α,15-diol from *Brachyglottis monroi*

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Abstract In a previous study, we reported that 13(*E*)-labd-13-ene-8α,15-diol (13E) possesses antiviral and anticancer activities. In this study, the anticancer and antimicrobial activities of 13(E) were evaluated against 4 cancer cell lines and 6 bacteria. 13(E) showed inhibitory effect on a variety of cancer cell lines. The IC_{50} values was 8.3–21.3 μg/mL. 13(E) was the most effective growth inhibitor of murine leukaemia cell lines P388, producing approximately 8.3 μg/mL of IC_{50} in the cytopathic effect (CPE) method. 13(E) also inhibited the growth of the gram-positive bacteria (*Staphylococcus aureus, Bacillus cereus* and *Listeria monocytogenes*) and gram-negative bacteria (*Vibrio parahaemolyticus, Escherichia coli* and *Salmonella enteritidis*) with a range of minimum inhibitory concentration (MIC) values from 0.092 to 0.598 mg/mL and gram-negative bacteria were more sensitive to the compound (MIC, 0.092 mg/mL).

Keywords anticancer · antimicrobial · 13(*E*)-Labd-13-ene-8α · 15-diol

Brachyglottis monroi (Hook.f.) B.Nord. (Asteraceae), previously *Senecio monroi*, is a shrub endemic to New Zealand (Allan, 1961; Connor and Edgar, 1987). We are not aware of any previous reports of compounds from *B. monroi,* but four antimicrobial diterpenes have been isolated from *B. bidwillii* (Bloor and Gainsford, 1993). Historically, *B. repanda* was traditionally used by the New Zealand Maori for treatment of sores and wounds (Riley, 1994). In our article, we reported that 13(*E*)-labd-13-ene-8α,15-diol (13E) isolated from *B. monroi* show anti-human rhinovirus 2 and 3 activities and anticancer activities on Carcinomic human alveolar basal epithelial (A549) and human larynx carcinoma (Hep2) cells (Choi et al., 2010).

In this study, we have analyzed the cytotoxicity of 13(E) on 4 cancer cells (murine leukemic cells P388, murine melanoma cell line B16-F10, human cervical carcinoma cell line KB and human colon cancer cells SNU-C4) and its antimicrobial activity against three gram-positive [*Staphylococcus aureus* (ATCC 13565), *Bacillus cereus* (ATCC 10702) and *Listeria monocytogenes* (ATCC 15313)] and three gram-negative [*Vibrio parahaemolyticus* (ATCC 17802), *Escherichia coli* (ATCC 25922) and *Salmonella enteritidis* (ATCC 13076)].

In our experiments, 13(E) was isolated from *Brachyglottis monroi* by previous report (Choi et al., 2010) and stock at –20°C. Sulforhodamine B (SRB) and mitomycin C were purchased from Sigma-Aldrich (USA). All other chemicals were a reagent grade. The murine leukemic cells P388, murine melanoma cell line B16-F10 and human cervical carcinoma cell line KB were purchased from the American Type Culture Collection (USA). Human colon cancer SNU-C4 cells were obtained from Korean Cell Line Bank (KCLB, Korea). The KB cells were cultured in DMEM with 4.5 g of glucose/L plus 10% fetal bovine serum, L-glutamine, penicillin (50 U/mL), and streptomycin (50 μg/mL). The other 3 cells were maintained in RPMI 1640 medium (Gibco, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco). Cells were grown in a humidified incubator at 37°C in an atmosphere of 5% CO_2 and 95% air.

For anticancer assay, a two-fold dilution series of the sample was incubated for 48 h with murine leukemia cells. The concentration of the sample required to inhibit cell growth to 50% of the growth

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Table 1 Inhibition of cancer cell proliferation by 13(*E*)-labd-13-ene-8α,15-diol

Drug \ Cell	IC_{50} (μg/mL)[1)]			
	P388	B16-F10	KB	SNU-C4
13(E)	8.3	16.3	20.4	21.3
Mitomycin C	0.6	1.1	0.9	1.4

[1)]IC_{50} value of 13(*E*)-labd-13-ene-8á,15-diol against each cancer cell lines, defined as the concentration that caused 50% inhibition of cell proliferation *in vitro*. The values were examined in three concentrations in triplicate experiments.

of a solvent control (IC_{50}) was determined using the absorbance obtained by staining with SRB as a previous described method (Lin et al., 1999).

Minimum inhibitory concentration (MIC) values were measured by the optical density method. The bacteria were then inoculated in microtubes (10 μL–10^8 CFU/mL) in 150 μL of Mueller-Hilton liquid medium (triptone 10 g/L, yeast extract 5 g/L, NaCl 5 g/L), containing the 13(E) at different concentrations, and incubated for 24 h at 37°C. Only *V. parahaemolyticus* was incubated in Mueller-Hilton medium with 3% NaCl. The optical density of the samples was measured at the beginning of the experiment and after 24 h incubation (Bio-Tec Instruments Inc., model EL800), at a wavelength of 490 nm. The following concentrations of the 13(E) were tested for the 5 bacterial species: 0, 0.046, 0.069, 0.092, 0.23, 0.46, 0.598, 0.690, 0.782, 0.92, 2.3 and 4.6 mg/mL. Bacterial growth was determined by computing the difference between the recorded optical density measurements at the start of the incubation and after 24 h.

As shown in Table 1, IC_{50} values of the cancer cells treated with 13(E) are 8.3 μg/mL for P388 cells, 16.3 μg/mL for B16-F10 cells, 20.4 μg/mL for KB cells and for 21.3 μg/mL for SNU-C4 cells, respectively. The 4 cancer cells treated with mitomycin C for 48 h showed IC_{50} values of 0.6–1.4 μg/mL against 4 cancer cells. Especially, 13(E) was the most effective growth inhibitor of P388 murine leukaemia cell lines, producing approximately 8.3 μg/mL of IC_{50}. The potencies (IC_{50}, 8.3–21.3 μg/mL) are similar to those reported (IC_{50}, 11.4 50 μg/mL) for 13(E) isolated from another plant, *Cistus creticus* (Cistaceae) (Dimas et al., 1998). It also observed that all the microorganisms tested were susceptible to the action of 13(E), with a range of MIC values from 0.092 to 0.598 mg/mL and gram-negative bacteria were more sensitive to the compound (Table 2). Results of MIC determinations for 13(E) obtained in this work are in accord with previous studies, the greater resistance of gram-negative bacteria to marjoram essential oil has been attributed in part to the great complexity of the double membrane-containing cell envelope of these microorganisms in contrast to the single membrane structures of gram-positive bacteria (Shapiro et al., 1994; Helander et al., 1998; Hammer et al., 1999; Velickovic et al., 2002; Kalemba and Kunicka, 2003; Bagamboula et al., 2004; Tepe et al., 2004).

Consequently, we demonstrated that the 13(E) had inhibitory activity against 5 cancer cell lines and 5 microorganisms. Although, further studies are needed, the use of 13(E) against microbial growth seems a valuable alternative as anticancer or antimicrobial compound.

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Table 2 Results of minimum inhibitory concentration (MIC) for 13(*E*)-labd-13-ene-8α,15-diol

	Bacterium	MIC			
		13(E) (mg/mL)	Tetracyclin (μg/mL)	Ciprofloxacin (μg/mL)	Imipenem (μg/mL)
Gram (+)	*Staphylococcus aureus*	0.598	0.782	2.300	0.598
	Bacillus cereus	0.460	2.300	0.920	0.069
	Listeria monocytogenes	0.460	0.920	2.300	0.460
Gram (−)	*Vibrio parahaemolyticus*	0.092	0.092	0.920	0.046
	Escherichia coli	0.092	0.460	0.782	0.092
	Salmonella enteritidis	0.092	0.690	0.690	0.069

The values were examined in three concentrations in triplicate experiments.

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