Screening for Biological Activity of Crude Extract and Bioactive Fractions from *Brachyglottis monroi*

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The effects of crude extract and bioactive fractions from *Brachyglottis monroi* on biological activity were investigated. The crude ethanol extract inhibited the growth of the Gram positive bacterium *Bacillus subtilis* (ATCC Strain number 19659, 1 mm zone at 150 μg/disk) and the dermatophyte *Trichophyton mentagrophytes* (ATCC 28185, 2 mm zone at 150 μg/disk), and toxic to P388 tumor cells (IC50 23.96 μg/ml at 75 μg/disk). Cytotoxic activity was strongly showed by Fr. 6 (P388 IC50 19.67 μg/ml at 75 μg/disk).

Key words : *Brachyglottis monroi, Bacillus subtilis, Trichophyton mentagrophytes,* P388, Cytotoxic activity

Introduction

Brachyglottis monroi (Hook. f) B. Nordenstam (Asteraceae compositae), previously *Senecio monroi*, is a shrub endemic to New Zealand.[1,2] *B. monroi* has been widely used in Maori traditional medicine for treatment of sores and wounds.[3] In this study, the antiviral and antimicrobial activities and cytotoxicity of crude ethanol extract from *B. monroi* were examined and have investigated their cytotoxic fractions.

Materials and Methods

1. General experimental procedures

All solvents were distilled before use. Removal of solvents from chromatography fractions were removed by rotary evaporation at temperature up to 40℃. Initial fractionation of crude plant extract using reverse phase column chromatography was performed with octadecyl-functionalized silica gel (C-19 Aldrich) as the adsorbent. Further column fractionation was performed using Davisil silica 60 Å (35-70 ㎛ silica gel, Allth) as adsorbent. TLC was carried out using Merck DC-plastikfolien Kieselgel 60 F254 visualized first with a UV lamp, then by dipping in a vanillin solution (1% vanillin, 1% H_2SO_4 in EtOH) followed by heating. NMR spectra of $CDCl_3$ solutions at 25℃ were recorded at 300 ㎒ for 1H on a varian VXR-300 spectrometer. Chemical shifts are given in ppm on the scale referenced to the solvent peaks of $CDCl_3$, 1H-NMR referenced to 7.25 ppm.

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· Received : 2003/03/12 · Revised : 2003/04/18 · Accepted : 2003/05/26

2. Plant material

Brachyglottis monroi was collected from the Dunedin Botanical Garden, Dunedin, New Zealand, in June 1998. This was identified by Dr. Glenny, Landcare Research, and a voucher specimen, OTA 980309-63, has been kept in the Otago University herbarium.

3. Preparation of the extract

Air-dried *Brachyglottis monroi* (26.88 g) was macrate in redistilled ethanol (200 ml) in a Waring Blender, and then filtered. the residual marc was reextracted in the same way with more ethanol (2 x 150 ml) and chloroform (100 ml). The combined filtrates were evaporated under reduced pressure to give a dark green gum (2.60 g) which was stored at 40C until tested.

4. Preparation of bioactive fractions

The crude ethanol extract (2.60 g) was subjected to flash column chromatographyon C 18 (10 g) with a H_2O : CH_3CN : $CHCl_3$ gradient. These fractions was stored at 4℃ until tested.

5. Screening for antiviral activity

The extract was applied (15 ㎕ of a 5 ㎎/㎖ solution) to

a small filter-paper disc, dried, and assayed for antiviral activity using Schroeder et al. methods.[4] The results were observed either cell death (cytotoxicity), inhibition of virus replication, no effect (i.e., all of the cells show viral infection), or a combination of all three. The results were noted as the approximate size of the circular zone, radiating from the extract sample, from 1+ to 4+ representing 25% through to whole well sized zones. The notation used is inhibition/ antiviral activity. The type of antiviral effect, indicated by a number after the size of the zone, was also considered important and may give some indication as to the mode of cytotoxic action.

6. Screening for antibacterial and antiyeast activities

Activity against the following bacterial strains and yeast was tested: multiresistant *B. subtilis* (ATCC 19659), and *Candida albicans* (ATCC 2091). Extracts were dissolved and diluted in an appropriate solvent (usually ethanol : water) to a concentration of 5 ㎎/㎖. Test plates are prepared from Mueller Hinton agar containing extract to give a final concentration of 100 ㎍ extract/㎖ agar. Activity growing cultures of the test strains were diluted in saline so as to deliver 10^4 colony forming units onto the test, control (solvent), and blank (agar only) plates with a multipoint inoculator. Inoculated plates were incubated overnight at 37℃. Growth on the blank and control plates was checked and, if satisfactory, growth on the test plates was scored for each test strain.

7. Screening for antifungal activity

Fungal spore suspensions of *Trichophyton mentagraphytes* (ATCC 28185) were applied to dextrose agar plates. Aliquots of the extract solutions were applied to filter paper discs, at 30 ㎍ extract/disc, and dired at 37℃ for two hours. These discs were applied to the agar plates, two per plate, and incubated at 28℃.

8. Screening for cytotoxic activity

This is a measure of the ability of a sample to inhibit the multiplication of murine leukaemia cells. The sample was dissolved in a suitable solvent, usually ethanol, at 5 ㎎/㎖, and 15 ㎕ of this solution was placed in the first well of a multiwell plate. Seven two-fold dilutions were made across the plate. After addition of the cell solution, the concentration range in the test wells was 25,000 down to 195 ng/㎖. After incubation for three days, the plates were read using an Elisa palte reader at 540 ㎚ wavelength. Automated reading of the plates was possible with the addition of a MTT tetrazolium salt (yellow color). Healthy cells reduce this salt to MTT formazan (purple color).

Results and Discussion

1. Biological screenings of extract

Brachyglottis monroi (Hook. f) B. Nordenstam(Asteraceae compositae) is a shrub endemic to New Zealand.[1,2] Foliage plant, collected from the Dunedin Botanical Gardens, gave a crude ethanol extract cytotoxic to P388 murine leukaemia (IC50 23.96 ㎍/ml) and BSC monkey kidney cells (25% of well at 75 ㎍/ml). Table 1 shows the weak antiviral activity against Herpes simplex and Polio (25% activity, @ 5 mg/ml at 75 ㎍/disk). The crude ethanol extract inhibited the growth of the Gram-positive and Gram-negative bacteria of the extract prepared from New Zealand medicinal plant, which have been used by Maori for treatment of sores and wounds.3 The activities are expresed by the diameter of the developed inhibition zones and compared with those of the widely antibious chloramph and nystatin. As indicated in Table 1, this crude extract inhibited the growth of the Gram-positive bacterium *Bacillus subtilis* (ATCC Strain number 19659, 1 mm zone at 150 ㎍/disk) and the dermatophyte *Trichophyton mentagrophytes* (ATCC Strain number 28185, 2 mm zone at 150 ㎍/disk). No activity was observed against the fungus *Candida albicans* at 150 ㎍/disk. This extract showed weaker antimicrobial activity than chloramph and nystatin (Table 1).

Table 1. Biological assays of ethanol extract from *B. monroi*

Extract	BSC	Cytotoxicity[a] *Herpes simplex*	*Polio*
		+	-
		Antimicrobial activity[b]	
	B. subtilis	*C. albicans*	*T. mentagrophytes*
Extract	Sm 1	-	Sm 2
Chloramph	Sm 12	0	0
Nystatin	0	Sm 12	Sm 8
		P388[c]	
Mitomycin C		659	
Extract		23956	

a : Cytotoxicity in antiviral assays. @ 5 mg/ml, 75 ㎍/disk; Zone of cytotoxic activity: + : 25% activity and - : not detected.. b : Width of zone of inhibition in mm; 150 ㎍/disk; - : not detected, 0: not determined.. c : Toxicity of sample to P388 tumor cells in ng/ml at 75 ㎍/disk.

2. Cytotoxic activity of bioactive fractions

The crude ethanol extract was fractioned into Fr. 1 - Fr. 8 using C-18 silica gel column chromatography as described previously.[5] Chromatography on C-18 (26.0 g) with a H_2O, MeCN, $CHCl_3$ gradient gave eight fractions. Among them, the fractions Fr. 4 - Fr. 7 are cytotoxic to P388 cells. The order of cytotoxic activity was showed Fr. 6 > Fr. 5 > Fr. 7 > Fr. 4 > Fr. 1 = Fr. 2 = Fr. 3 = Fr. 8 (Table 2). Reverse-phase flash column chromatography concentrated the cytotoxic activity in fractions eluted with 1 : 9 H_20 : MeCN which gave a major active (245 mg, P388 IC_{50} 1967 ng/ml at 75 ㎍/disk). This

bioactive fraction was cytotoxic to P388 cells (P388 IC_{50} 8049 ng/ml at 30 μg/disk; P388 IC_{50} > 6250 ng/ml at 7.5 μg/disk).

Table 2. In vitro cytotoxic activities of bioactive fractions on P388 tumor cell lines by MTT assay.[a]

Fraction No.	Eluent	Volume (ml)	Mass (mg)	IC_{50} (ng/ml)[b]
1	H_2O, 9:1 H_2O/CH_3CN	90	133	) 62500
2	9:1 H_2O/CH_3CN	30	140	) 62500
3	3:1 H_2O/CH_3CN	60	139	) 62500
4	1:1 H_2O/CH_3CN	60	145	17914
5	1:3 H_2O/CH_3CN	60	285	9730
6	1:9 H_2O/CH_3CN	60	245	1967
7	CH_3CN	60	222	10312
8	3:1 $CH_3CN/CHCl_3$ $CHCl_3$, 1:1 $CHCl_3$/EtOH EtOH	240	1152	) 62500

a : Each fraction was examined in eight concentrations in triplicated experiments. b : IC50 represents the concentration of a fraction required for 50% inhibition of cell growth. Mitomycin C was used as control and exhibited an IC_{50} 52.3 ng/ml. Toxicity of sample to P388 tumor cells in ng/ml at 75 μg/disk.

The ^{1}H-NMR spectrum of the Fr. 6 fraction showed signals due to an olefinic group (δ5.38 ppm), methylene protons and five methyl groups (δ 0.72, 0.73. 0.80 1.07 and 1.63 ppm). By ^{1}H-NMR spectrum analysis, the main components of Fr. 6 were identified as labdane-type diterpenes. In this study, *B. monroi* has proved to inhibited antiviral, antimicrobial activities and cytotoxicity. The separation of the main components from the bioactive fraction Fr. 6 of *B. monroi* extract need to be studied further and the results will be discussed elsewhere.

Acknowledgements

We thank the Dunedin City Council for permission to collect; N. Brennan and E. Burgess for collection; G. Ellis for biological assays. This work was supported by Wonkwang Health Science College and Brain Korea 21 Project.

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