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Screening for Biological Activity of Crude Extracts from Medicinal plants

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ABSTRACT The biological effects of the crude extracts from medicinal plants, *Brachyglottis monroi* and *Trichocolea hatcheri* were investigated. The crude ethanol extract inhibited the growth of the Gram positive bacterium *Bacillus subtilis* (ATCC 19659, 1 mm inhibition zone at 150 μg/disc) and the dermatophyte *Trichophyton mentagrophytes* (ATCC 28185, 6 mm inhibition zone at 150 μg/disc), and was toxic to P388 murine leukaemia cells (IC_{50} 23.96 μg/mL at 75 μg/disc). *B. monroi* ethanol extract showed stronger antiviral activity than that of *T. hatcheri* against *Herpes simplex* Type I *virus* (ATCC VR 733) and *Polio* Type I *virus* (Pfizer vaccine strain) (50% activity, @ 5 mg/ml at 150 μg/disc). The crude ethanol extract of *T. hatcheri* showed stronger antimicrobial activity than that of *B. monroi*. However, this extract was inactive against P388 murine leukaemia cells.

Key words *Brachyglottis monroi*, *Trichocolea hatcheri*, *Bacillus subtilis*, *Trichophyton mentagrophytes*, P388, Cytotoxicity

I. INTRODUCTION

There is world-wide interest in bioactive natural products from plant as new agents for controlling human and agricultural diseases and pests. Research groups in many countries are engaged in the screening of plant extract. Liverworts are closely related to mosses, and the two groups together form a large and important division of the plant kingdom, technically known as the Bryophytes[1)]. Liverworts of the genus *Trichocolea* (family Trichocoleaceae) are a treasury of isoprenyl phenyl ethers. *Trichocolea hatcheri* Hodgs, which grows throughout New Zealand, is distinguished from *T. mollissima* by its smaller size, dark green color, and prostrate habit[2)].

A chemical abstracts search of *Trichocolea* revealed that only *T. tomentella*[3-5)], *T. lanata*[3)], *T. mollisima*[3,6)], *T. hatcheri*[7)] and *T. pluma*[8)] have been studied for their chemical constituents.

Brachyglottis monroi (Hook. f) B. Nordenstam (Asteraceae compositae), previously *Senecio monroi*, is a shrub endemic to New Zealand[9,10)]. *B. monroi* has been widely used in Maori traditional medicine for treatment of sores and wounds[11)].

In this study, the antiviral, antimicrobial activities and cytotoxicity of crude ethanol extracts from medicinal plants were examined.

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MATERIALS AND METHODS

1. General experimental procedures

All solvents were distilled before use and were removed by rotary evaporation at temperatures up to 35ºC. Octadecyl functionalized silica gel (C 18, Aldrich) was used for reversed-phase flash chromatography, and Davisil, 35-70 μm, 150 Å was used for Si gel flash chromatography. Preparative silica gel TLC was carried out using Merck DC-plastikfolien Kieselgel 60 F_{254}, visualized with an UV lamp then by dipping in a vanillin solution (1% vanillin, 1% H_2SO_4 in EtOH) and heating.

2. Plant material

Trichocolea hatcheri (*T. hatcheri*) was collected from a steep earth bank in the Morrisons Creek area, Dunedin, New Zealand, in Fabruary 1996 [University of Otago Herbarium (OTA) specimen no. 048094].

Brachyglottis monroi (*B. 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 medicinal plants were marcate in redistilled ethanol in a Waring Blender, and then filtered. The residual marc was re-extracted in the same way with more ethanol and chloroform. The combined filtrates were evaporated under

reduced pressure to give a dark green gum, which was stored at 4°C until tested.

4. Screening for antiviral activity

The extract was applied (15 L of a 5 mg/mL solution) to a small filter-paper disc, dried, and assayed for antiviral activity using Schroeder *et al.*'s[12] methods. The results were observed 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.

5. Screening for antibacterial and anti-yeast activities

Activity against the following bacterial strains and yeast was tested: multi-resistant *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 mg/mL. Test plates are prepared from Müller Hinton agar containing extract to give a final concentration of 100 µg extract/mL 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°C. Growth on the blank and control plates were checked and, if satisfactory, growth on the test plates was scored for each test strain as follows: (−) inhibition, no reduction in growth compared with the control, (+) inhibition, no growth.

6. Screening for antifungal activity

Activity against the following fungal strain was tested: *Trichophyton mentagraphytes* [(ATCC 28185) local strain]. Fungal spore suspensions of the test organisms were applied to dextrose agar plates. Aliquots of the extract solutions were applied to filter paper discs, at 15 µg extract/disc, and dried at 37°C for two hours. These discs were applied to the agar plates, two per plate, and incubated at 28°C.

7. Screening for cytotoxic activity against animal cell

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 mg/mL, and 15 µL 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/mL. After incubation for three days, the plates were read using an ELISA palte reader at 540 nm 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 screening of the crude extract of *T. hatcheri*

Trichocolea hatcheri Hodgs (family Trichocoleaceae) grows throughout New Zealand. Foliage plant collected from a steep earth bank in the Morrisons Creek area in Dunedin.

An extract of *T. hatcheri* was prepared by grinding dried plant material and extracting separately with ethanol then chloroform. The two extracts were combined, as their ^{1}H-NMR spectra were similar. A crude extract was cytotoxic to P388 murine leukemia

(IC_{50} > 12,500 µg/mL) and BSC monkey kidney cells (50% of well at 150 µg/mL). Table 1 shows the mediocre antiviral activity against *Herpes simplex* Type I *virus* (ATCC VR 733) and *Polio* Type I *virus* (Pfizer vaccine strain) (50% activity, @ 5 mg/mL at 150 µg/disc). The crude extract inhibited the growth of the Gram-positive bacteria and fungus of the extract prepared from New Zealand medicinal plant. As indicated in Table 1, this crude extract inhibited the growth of the Gram-positive bacterium *Bacillus subtilis* ATCC 19659 (1 mm inhibition zone at 150 µg/disc) and the dermatophyte *Trichophyton mentagrophytes* ATCC 28185 (6 mm inhibition zone at 150 µg/disc). No activity was observed against the fungus *Candida albicans* at 150 µg/disc. This extract showed weaker antimicrobial activity than chloramphenicol and nystatin (Table 1). However, this crude extract showed stronger antimicrobial activity than the crude extract of *B. monroi* and this extract was inactive against P388 murine leukaemia cells.

Table 1. **Biological assays of the crude extract from *T. hatcheri***

Extract	Cytotoxicity		
	BSC[a]	*Herpes simplex virus*[b]	*Polio virus*[b]
	++	++	++
	Antimicrobial activity[c]		
	B. subtilis	*C. albicans*	*T. mentagrophytes*
Extract	SM 1	-	HM 2
Chloramphenicol	SM 12	0	0
Nystatin	0	SM 11	HM 8
	P388 cytotoxicity		
Mitomycin C	59.7[d]		
Extract	> 12,500[e]		

[a]% of well showing cytotoxic effects. @ 5 mg/mL, 150 µg/disc; ++: 50% activity.
[b]Cytotoxicity in antiviral assays. @ 5 mg/mL, 150 µg/disc; Zone of cytotoxic activity: ++: 50% activity.
[c]Width of zone of inhibition in mm; 150 µg/disc; -: no reduction in growth, 0: not determined. Chloramphenicol; 30 mcg/disc, Nystatin; 100 unit/disk. SM; Sharp margin, HM; hazy margin, numbers refer to zone of inhibition (mm)
[d]Toxicity of sample to P388 murine leukaemia cells in ng/ml at 0.075 µg/disc. P388; Concentration of the sample required to inhibit cell growth to 50% of a solvent control.
[e]Toxicity of sample to P388 murine leukaemia cells in ng/mL at 75 µg/disc

2. Biological screening of the crude extract of *B. monroi*

Brachyglottis monroi (Hook. f) B. Nordenstam (Asteraceae compositae) is a shrub endemic to New Zealand[9,10]. Foliage plant collected from the Dunedin Botanical Gardens. An extract of *B. monroi* was prepared by grinding dried plant material and extracting separately with ethanol then chloroform. The two extracts were combined, as their ^{1}H-NMR spectra were similar. A crude extract was cytotoxic to P388 murine leukaemia (IC_{50} 23.96 μg/mL) and BSC monkey kidney cells (25% of well at 150 μg/mL). However, this crude extract is stronger cytotoxic activity than the extract of *B. monroi*. Table 2 shows the weak antiviral activity against *Herpes simplex* Type I *virus* (ATCC VR 733) and *Polio* Type I *virus* (Pfizer vaccine strain) (25% activity, @ 5 mg/mL at 75 μg/disc). The crude extract inhibited the growth of the Gram-positive bacteria and fungus of the extract prepared from New Zealand medicinal plant, which have been used by Maori for treatment of sores and wounds[11]. The activities are expressed by the diameter of the developed inhibition zones and compared with those of the widely antibious chloramphenicol and nystatin. As indicated in Table 2, this crude extract inhibited the growth of the Gram-positive bacterium *Bacillus subtilis* (ATCC 19659, 1 mm inhibition zone at 150 μg/disc) and the dermatophyte *Trichophyton mentagrophytes* (ATCC 28185, 2 mm inhibition zone at 150 μg/disc). No activity was observed against the fungus Candida albicans at 150 μg/disk. This extract showed weaker antimicrobial activity than chloramphenicol and nystatin (Table 2).

Table 2. Biological assays of the crude extract from *B. monroi*

	Cytotoxicity		
Extract	BSC[a]	*Herpes simplex virus*[b]	*Polio virus*[b]
	+	+	0
	Antimicrobial activity[c]		
	B. subtilis	*C. albicans*	*T. mentagrophytes*
Extract	SM 1	-	SM 2
Chloramphenicol	SM 12	0	0
Nystatin	0	SM 12	SM 8
	P388 cytotoxicity		
Mitomycin C	65.9[d]		
Extract	23,956[e]		

[a]% of well showing cytotoxic effects. @ 5 mg/mL, 150 μg/disc; +: 25% activity and 0: not determined.
[b]Cytotoxicity in antiviral assays. @ 5 mg/mL, 150 μg/disc; Zone of cytotoxic activity: +: 25% activity and -: not detected.
[c]Width of zone of inhibition in mm; 150 μg/disc; -: no reduction in growth, detected, 0: not determined. Chloramphenicol; 30 mcg/disc, Nystatin; 100 unit/disc. SM; Sharp margin, numbers refer to zone of inhibition (mm)
[d]Toxicity of sample to P388 murine leukaemia cells in ng/mL at 0.075 μg/disc. P388; Concentration of the sample required to inhibit cell growth to 50% of a solvent control.
[e]Toxicity of sample to P388 murine leukaemia cells in ng/mL at 150 μg/disc

In conclusion, these crude extracts inhibited the growth of the Gram positive bacterium *Bacillus subtilis* ATCC 19659 (1 mm inhibition zone at 150 μg/disc) and the dermatophyte *Trichophyton mentagrophytes* ATCC 28185 (6 mm inhibition zone at 150 μg/disc), and were toxic to P388 murine leukaemia cells (IC_{50} 23.96 μg/mL at 75 μg/disc). *B. monroi* ethanol extract showed stronger antiviral activity than that of *T. hatcheri* against *Herpes simplex* Type I *virus* (ATCC VR 733) and *Polio* Type I *virus* (Pfizer vaccine strain) (50% activity, @ 5 mg/ml at 150 μg/disc). The crude ethanol extract of *T. hatcheri* showed stronger antimicrobial activity than that of *B. monroi*. However, this extract was inactive against P388 murine leukaemia cells. Further research is needed to the separation of the main bioactive components from the extracts of plants and the results will be discussed elsewhere.

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생약추출물로부터 생리활성의 검색

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뉴질랜드의 자생식물인 *Brachyglottis monroi* 와 *Trichocolea hatcheri*의 에탄올추출물에 대한 생리활성을 조사한 결과, 그람음성균인 *Bacillus subtilis* (ATCC 19659, 1 mm inhibition zone at 150 μg/disc)와 피부곰팡이균인 *Trichophyton mentagrophytes* (ATCC 28185, 6 mm inhibition zone at 150 μg/disc)에 대한 억제효과가 관찰되었으며, 이들 추출물은 P388 murine leukaemia cells (IC_{50} 23.96 μg/mL)에서도 세포독성이 나타났다. *B. monroi*의 에탄올추출물의 항바이러스효과는 *T. hatcheri*의 에탄올추출물보다 강한 활성이 관찰되었으며, *T. hatcheri*의 에탄올추출물의 항균효과는 *B. monroi*의 에탄올추출물보다 강한 활성을 나타났다. 그렇지만 P388 murine leukaemia cells (IC_{50} > 12,500 μg/mL)에 대한 세포독성은 아주 미미한 것으로 나타났다.