Antifungal Activity of Chloroform Extract from *Lepidolaena Taylorii* on the Dermatophytic Fungus *Trichophyton mentagrophytes*

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The effects of chloroform extract from *Lepidolaena taylorii (L. taylorii)* on antifungal activity were investigated. The crude chloroform extract of *L. taylorii* inhibited the growth of the Gram positive bacteria *Bacillus subtilis* ATCC 19659, (5 mm inhibition zone at 150 μg/disc) and the dermatophytic fungus *Trichophyton mentagrophytes* ATCC 28185, (6 mm inhibition zone at 150 μg/disc), and cytotoxic to P388 murine leukaemia cells ATCC CCL 46 P388D1, (IC_{50} 405.0 μg/mL at 150 μg/disc) and cytotoxic to BSC monkey kidney cells (@ 5 mg/mL, 150 μg/disc; ++++: 100% activity). We suppose that this crude chloroform extract of *L. taylorii* is the strong antimicrobial and cytotoxic activities.

Key words : *Lepidolaena taylorii*, *Bacillus subtilis*, *Trichophyton mentagrophytes*, antifungal activity, P388 murine leukaemia cells, BSC monkey kidney cells

Introduction

Liverworts are the only class of the Bryophytes that contain complex oil bodies[1)], and so are capable of synthesising a vast range of lipophilic aromatic and terpenoid compounds; it is perhaps because of this that liverworts have been more thoroughly investigated than mosses or hornworts for their rich and varied chemistry.[2,3)] Eight liverwort species have been assigned to the genus *Lepidolaena* (family Lepidolaenaceae), with *L. taylorii* being the most common of these in New Zealnd[4)]. This endemic species is widely distributed throughout the country, especially in wet forest[5)]. The only other report on *Lepidolaena* chemistry is of several sesquiterpenes, including a new bergamotane diacetate, from another New Zealand species *L. clavigera* (Hook.) Dum. ex Trev[6)]. In this study, the biological activity of the crude chloroform extract from *L. taylorii* was examined.

Materials and Methods

1. General experimental procedures

All solvents were distilled before use and were removed by rotary evaporation at temperatures up to 35℃. Preparative silica gel TLC was carried out using Merck DC-plastikfolien Kieselgel 60 F254, visualized with an UV lamp then by dipping in a vanillin solution (1% vanillin, 1% H_2SO_4 in EtOH) and heating.

2. Plant materials

Lepidolaena taylorii (L. taylorii) was collected from tree trunks in the Cascade Valley, on the West Coast of the South Island of New Zealand, in March 1997. A voucher specimen, collection code 970321-07, has been deposited in the University of Otago Herbarium (OTA).

3. Preparation of the extract

Ground material (80 g) was extracted with chloroform (1 L) in a Soxhlet apparatus for 24 hrs, then the extract was dried to give a green gum (4 g). Two further 24 hrs extractions yielded a total crude extract of 13 g. which was stored at 4℃ until tested.

4. Screening for antiviral activity

The extract was applied (30 μL of a 5 mg/mL solution) to a small filter-paper disc, dried, and assayed for antiviral activity using Schroeder et al.s methods[7)]. The results were observed either cell death (cytotoxicity), inhibition of virus

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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 antiyeast activities

Activity against the following bacterial strains and yeast was tested: multiresistant *Bacillus subtilis*(ATCC 19659), *Staphylococcus aureus*(ATCC 6538P) *Escherichia coli*(ATCC 25922), and *Candida albicans*(ATCC 14053). Extracts were dissolved and diluted in an appropriate solvent (usually ethanol : water) to a concentration of 5 mg/mL. Test plates are prepared from Mueller 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℃. Growth on the blank and control plates was 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. Solutions of compound for assay were dried onto 6 mm filter paper disks, which were then placed onto seeded agar Petri dishes and incubated. Activity was observed as a zone of inhibition around the disk, with its width recorded from the edge of the disk in mm. HM and SM refer to the observed margin surrounding the zone of inhibition. (H= hazy, S= sharp).

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 oraganisms were applied to dextrose agar plates. Aliquots of the extract solutions were applied to filter paper discs, at 30 μg extract/disc, and dired at 37℃ for two hours. These discs were applied to the agar plates, two per plate, and incubated at 28℃.

7. 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 mg/mL, and 30 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

L. taylorii (Gott.) Trev. (family Lepidolaenaceae) is widely distributed throughout New Zealnd, especially in wet forst[5]. This plant collected from tree trunks in the Cascade Valley, on the West Coast of the South Island of New Zealand. An crude extract of *L. taylorii* was prepared by grinding dried plant material and extracted with chloroform. A crude extract was cytotoxic to P388 murine leukaemia cells ATCC CCL 46 P388D1, (IC_{50} 405.0 μg/mL) and cytotoxic to BSC monkey kidney cells (@ 5 mg/mL, 150 μg/disc; ++++: 100% activity). The main cytotoxic components were secokauranoids. Table 1 does not show the antiviral activity against *Herpes simplex* Type I *virus* (ATCC VR 733) and *Polio* Type I *virus* (Pfizer vaccine strain) (@ 5 mg/mL at 150 μg/disc).

Table 1. Biological activities of the crude extract from *L. taylorii*

Assay	Tested material				
	Crude extract	Chloramphenicol	Nystatin	Gentamycin	Mitomycin C
Cytotoxicity [a]	++++				
BSC-1 cells					
P388					
IC_{50}	405.0[b]				59.7[c]
Antiviral activity[d]					
Herpes simplex virus	-				
Polio virus	-				
Antimicrobial activity [e]					
B. subtilis	SM 5	SM 12	0	0	
E. coli	-	0	0	SM 9	
C. albicans	-	0	SM 11	0	
T. mentagrophytes	HM 6	0	HM 8	0	

[a]% of well showing cytotoxic effects, with virus growing in cytotoxic zone. @ 5 mg/mL, 150 μg/disc: ++++: 100% activity. BSC-1 cells: African green monkey kidney cells. [b]Toxicity of sample to P388 murine leukaemia cells (ATCC CCL 46 P388D1) in ng/mL at 150 μg/disc. [c]Toxicity of sample to P388 murine leukaemia cells (ATCC CCL 46 P388D1) in ng/mL at 0.075 μg/disc. P388 : Concentration of the sample required to inhibit cell growth to 50% of a solvent control. [d]Antiviral assays. @ 5 mg/mL, 150 μg/disc: Zone of cytotoxic activity: -: no activity. [e]Width of zone of inhibition in mm: 150 μg/disc: -: no reduction in growth, 0: not determined. Chloramphenicol: 30 μg/disc, Nystatin: 100 unit/disk. SM: Sharp margin, HM: Hazy margin, numbers refer to zone of inhibition (mm)

The crude extract inhibited the growth of the Gram-positive bacterium and fungi of the extract prepared

from *L. taylorii*. As indicated in Table 1, this crude extract inhibited the growth of the Gram-positive bacteria *Bacillus subtilis* ATCC 19659, (5 mm inhibition zone at 150 μg/disc) and the dermatophytic fungus *Trichophyton mentagrophytes* ATCC 28185, (6 mm inhibition zone at 150 μg/disc). No activity was observed against the fungus *Candida albicans* (ATCC 14053) and the bacterium *Escherichia coli* (ATCC 25922) at 150 μg/disc. This extract showed weaker antimicrobial activity than chloramphenicol and nystatin (Tables 1 and 2)[8].

Table 2. List of microorganisms used for antimicrobial susceptibility test.

Gram-positive bacteria *Bacillus subtilis*	ATCC 19659
Gram-negative bacterium *Escherichia coli*	ATCC 25922
Fungi *Candida albicans* *Trichophyton mentagrophytes*	ATCC 14053 ATCC 28185

In conclusion. the crude chloroform extract of *L. taylorii* inhibited the growth of the Gram positive bacteria *Bacillus subtilis* ATCC 19659, (5 mm inhibition zone at 150 μg/disc) and the dermatophytic fungus *Trichophyton mentagrophytes* ATCC 28185, (6 mm inhibition zone at 150 μg/disc), and cytotoxic to P388 murine leukaemia cells ATCC CCL 46 P388D1, (IC_{50}=405.0 μg/mL at 150 μg/disc) and cytotoxic to BSC monkey kidney cells (@ 5 mg/mL, 150 μg/disc; ++++: 100% activity). We suppose that this crude chloroform extract of *L. taylorii* is the strong antimicrobial and cytotoxic activities. The separation of the main bioactive components from the extracts of plants need to be studied further and the results will be discussed elsewhere.

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