

# Antifungal Activity of Ethanol Extract from *Lepidolaena clavigera* on the Dermatophytic Fungus

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The effects of ethanol extract from *Lepidolaena clavigera* (*L. clavigera*) on antifungal activity were investigated. The crude ethanol extract of *L. clavigera* inhibited the growth of the Gram positive bacterium *Bacillus subtilis* ATCC 19659, (1 mm inhibition zone at 150 µg/disc) and the dermatophytic fungus *Trichophyton mentagrophytes* ATCC 28185, (2 mm inhibition zone at 150 µg/disc), and cytotoxic to P388 murine leukaemia cells ATCC CCL 46 P388D1, (IC<sub>50</sub> >12,500 µg/mL at 150 µg/disc) and cytotoxic to BSC monkey kidney cells (@ 5 mg/mL, 150 µg/disc; ++: 50% activity). We suppose that this crude ethanol extract of *L. clavigera* is the antifungal activity.

Key words : *Lepidolaena clavigera*, *Bacillus subtilis*, *Trichophyton mentagrophytes*, antifungal activity

## Introduction

Liverworts are the only class of the Bryophytes that contain complex oil bodies<sup>1)</sup>, 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<sup>2,3)</sup>.

Liverwort species have been assigned to the genus *Lepidolaena* (family Lepidolaenaceae), with *L. clavigera* being the most common of these in New Zealand. This endemic species is widely distributed throughout the country in rain forest and is common on the West Coast of the South Island and in Fiordland<sup>4)</sup>. The *Lepidolaena* chemistry is of several sesquiterpenes, including a new bergamotane diacetate, from another New Zealand species *L. clavigera* (Hook.) Dum. ex Trev<sup>5)</sup>. At least two other *Lepidolaena* species are found in New Zealand, *L. clavigera* (Hook.) Dum. ex Trev. and *L. hodgsoniae* Grolle, plus a related species, *Gackstroemia weindorferi* (Herz.) Grelle<sup>4)</sup>. Shin et al.<sup>6)</sup> reported that the effects of chloroform extract from *L. taylorii* inhibited the growth of the Gram positive bacterium *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<sub>50</sub> 405.0 µg/mL at 150 µg/disc) and cytotoxic to BSC monkey kidney cells (@ 5 mg/mL, 150 µg/disc; ++++: 100% activity).

In this study, the biological activity of the crude ethanol extract from *L. clavigera* 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°C. Preparative silica gel TLC was carried out using Merck DC-plastikfolien Kieselgel 60 F<sub>254</sub>, visualized with an UV lamp then by dipping in a vanillin solution (1% vanillin, 1% H<sub>2</sub>SO<sub>4</sub> in EtOH) and heating.

### 2. Plant materials

*Lepidolaena clavigera* (*L. clavigera*) was collected from coast rain forest on the South Island of New Zealand, in June 1993. A voucher specimen, collection code 930609-03, has been deposited in the University of Otago Herbarium (OTA).

### 3. Preparation of the extract

Air-dried *L. clavigera* was macerate in redistilled ethanol in a Waring Blender, and then filtered. the residual marc was

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· Received : 2005/05/26 · Revised : 2006/12/20 · Accepted : 2006/01/20

reextracted in the same way with ethanol. 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 (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<sup>7</sup>. 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<sup>+</sup> to 4<sup>+</sup> 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), 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<sup>4</sup> 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 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.

#### 6. Screening for antifungal activity

Activity against the following fungal strain was tested *Trichophyton mentagrophytes* (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 30 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

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. clavigera* (Hook.) Dum. ex Trev. (family Lepidolaenaceae) is widely distributed throughout New Zealand in rain forest<sup>4</sup>. This plant collected from the high rainfall area on the West Coast of the South Island of New Zealand and in Fiordland. An crude extract of *L. clavigera* was prepared by grinding dried plant material and extracted with ethanol. A crude extract was cytotoxic to P388 murine leukaemia cells ATCC CCL 46 P388D1, (IC<sub>50</sub> > 12,500 µg/mL) and cytotoxic to BSC monkey kidney cells (++; 50% activity @ 5 mg/mL at 150 µg/disc). This crude extract showed lower cytotoxic activity than the chloroform extract of *L. taylorii*. because of clavigerins<sup>8</sup>.

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

Assay	Tested material				
	Crude extract	Chloramphenicol	Nystatin	Gentamycin	Mitomycin C
Cytotoxicity <sup>a</sup>					
BSC-1 cells	++				
P388					
IC <sub>50</sub>	25,000 <sup>b</sup>				59.7 <sup>c</sup>
Antiviral activity <sup>d</sup>					
<i>Herpes simplex virus</i>	-				
<i>Polio virus</i>	-				
Antimicrobial activity <sup>e</sup>					
<i>B. subtilis</i>	SM 1	SM 12	0	0	
<i>E. coli</i>	-	0	0	0	
<i>S. aureus</i>	-	0	0	SM9	
<i>C. albicans</i>	-	0	SM 11	0	
<i>T. mentagrophytes</i>	SM 2	0	HM 8	0	

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

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). The crude extract inhibited the growth of the Gram-positive bacterium and fungus of the extract prepared from *L. clavigera*. 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 dermatophytic fungus *Trichophyton mentagrophytes* ATCC 28185, (2 mm inhibition zone at 150 µg/disc). No activity was observed against the fungus *Candida albicans* (ATCC 14053) and against the bacteria *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 6538P) at 150 µg/disc. This extract showed weaker antimicrobial activity than chloramphenicol and nystatin (Tables 1 and 2)<sup>9</sup>.

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

Gram-positive bacteria	
<i>Bacillus subtilis</i>	ATCC 19659
<i>Staphylococcus aureus</i>	ATCC 6538P
Gram-negative bacterium	
<i>Escherichia coli</i>	ATCC 25922
Fungi	
<i>Candida albicans</i>	ATCC 14053
<i>Trichophyton mentagrophytes</i>	ATCC 28185

In conclusion the crude ethanol extract of *L. clavigera* inhibited the growth of the Gram positive bacterium *Bacillus subtilis* ATCC 19659, (1 mm inhibition zone at 150 µg/disc) and the dermatophytic fungus *Trichophyton mentagrophytes* ATCC 28185, (2 mm inhibition zone at 150 µg/disc), and cytotoxic to P388 murine leukaemia cells ATCC CCL 46 P388D1, (IC<sub>50</sub> 25,000 µg/mL at 150 µg/disc) and cytotoxic to BSC monkey kidney cells (@ 5 mg/mL, 150 µg/disc; ++: 50% activity). We suppose that this crude ethanol extract of *L. clavigera* is antimicrobial and weak 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.

## Acknowledgement

We thank Dr. N. B. Perry at the Plant Extracts Research Unit, New Zealand Institute for Crop & Food Research Ltd, Department of Chemistry, University of Otago in New Zealand. This work was supported by Wotkwang University in 2005.

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