Antifungal Activity of the Crude Extract from *Quintinia acutifolia* on the Dermatophytic fungus

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The crude extract of *Quintinia acutifolia* Kirk inhibited the growth of the Gram positive bacterium *Bacillus subtilis* ATCC 19659, (3 mm inhibition zone at 150 µg/disc) and the dermatophytic fungus *Trichophyton mentagrophytes* ATCC 28185, (3 mm inhibition zone at 150 µg/disc), and cytotoxic to P388 murine leukaemia cells ATCC CCL 46 P388D1, (IC_{50} 50,000 µg/mL at 150 µg/disc). However, *Candida albicans* (ATCC 14053) did not observed the antimicrobial activity and the cytotoxic activity to BSC monkey kidney cells (@ 5 mg/mL, 150 µg/disc).

Key words : *Quintinia acutifolia, Bacillus subtilis, Trichophyton mentagrophytes*, antifungal activity, cytotoxic activity

Introduction

The genus *Quintinia* A. DC. (family Grossulariaceae) contains around 15 - 20 species of trees or shrubs, found in New Zealand, Australia, the Phillippines, and Papua New Guinea[1]. The three species endemic to New Zealand are *Quintinia acutifolia* Kirk (*Q. acutifolia*), *Q. elliptica* Hook. f., and *Q. serrata* Cunn[1,2]. *Q. acutifolia* grows in lowland and higher mountain forests and in found in the North Island and northern South Island[2]. *Q. acutifolia* and *Q. serrata* have similar morphological features, but *Q. serrata* leaves are more coarsely serrated and are shorter and narrower (5 - 15 cm long, 1 - 3 cm wide)[3]. The leaves are oblong in shape and greenish yellow, blotched with dark green and red. They are (6 - 12.5 cm long, 2.5 cm wide) narrower and the margins are more coarsely serrated than the leaves of *Q. acutifolia*[1]. The *Quintinia* chemistry is of several flavonols, iridoids, proanthocyanidines and alkaliods from the plants of Escalloniaceae[4,5]. In this study, the biological activity of the crude extract from *Q. acutifolia* 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 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 materials

Leaves of *Quintinia acutifolia*(*Q. acutifolia*) were collected in June 2001 from the Botanic Gardens, Dunedin, New Zealand, and were identified by A. Evans. Voucher specimens (010615-01) have been deposited in the Plant Extracts Unit Herbarium, Chemistry Department, University of Otago, Dunedin, New Zealand.

3. Preparation of the extract

Air-dried *Q. acutifolia* leaves (100.1 g) were ground in a in a Waring Blender, with ethanol (3 x 500 ml) and chloroform (500 ml). The combined extracts were filtrated, and the solvent was evaporated in vacuo. This afforded a brown-green solid mass (20.56 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[6]. 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

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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), 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

Q. acutifolia grows in lowland and higher mountain forests and in found in the North Island and northern South Island[2]. The leaves are oblong in shape and greenish yellow, blotched with dark green and red. An crude extract of *L. clavigera* was prepared by grinding dried plant material and extracted with ethanol and chloroform. A crude extract was cytotoxic to P388 murine leukaemia cells ATCC CCL 46 P388D1, (IC_{50} 50 μg/mL) and not cytotoxic to BSC monkey kidney cells (@ 5 mg/mL at 150 μg/disc). The main cytotoxic components were biflavonoids. 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 *Q. acutifolia*. As indicated in Table 1. this crude extract inhibited the growth of the Gram-positive bacterium *Bacillus subtilis* ATCC 19659, (3 mm inhibition zone at 150 μg/disc) and the dermatophytic fungus *Trichophyton mentagrophytes* ATCC 28185, (3 mm inhibition zone at 150 μg/disc). No activity was observed against the fungus *Candida albicans* (ATCC 14053) at 150 μg/disc. This extract showed weaker antimicrobial activity than chloramphenicol and nystatin (Tables 1 and 2)[7].

Table 1. Biological activities of the crude extract from *Quintinia acutifolia*

Assay	Tested material			
	Crude extract	Chloramphenicol	Nystatin	Mitomycin C
Cytotoxicity[a]				
BSC-1 cells		-		
P388				
IC_{50}	50,000[b]			60.0[c]
Antiviral activity[d]				
Herpes simplex virus	-			
Polio virus	-			
Antimicrobial activity[e]				
B. subtilis	SM 3	SM 12		0
C. albicans	-	0		SM 11
T. mentagrophytes	SM 3	0		HM 8

[a]% of well showing cytotoxic effects, with virus growing in cytotoxic zone. @ 5 mg/mL, 150 μg/disc: -: no 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 0.06 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)

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

Gram-positive bacterium	
Bacillus subtilis	ATCC 19659
Fungi	
Candida albicans	ATCC 14053
Trichophyton mentagrophytes	ATCC 28185

In conclusion the crude extract of *Q. acutifolia* inhibited the growth of the Gram positive bacterium *Bacillus subtilis* ATCC 19659, (3 mm inhibition zone at 150 μg/disc) and the dermatophytic fungus *Trichophyton mentagrophytes* ATCC 28185, (3 mm inhibition zone at 150 μg/disc), and cytotoxic to P388 murine leukaemia cells ATCC CCL 46 P388D1, (IC_{50} 50,000 μg/mL at 150 μg/disc). However, *Candida albicans* (ATCC 14053) did not observed the antimicrobial activity and the cytotoxic activity to BSC monkey kidney cells (@ 5 mg/mL, 150 μg/disc). We suppose that this crude extract of *Q. acutifolia* 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.

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