INSTITUTE OF APPLIED SCIENCES

THE UNIVERSITY OF THE SOUTH PACIFIC

ANTIMICROBIAL TESTING OF SELECTED FIJIAN PLANTS - PART 2

IAS TECHNICAL REPORT NO. 2002/01

by

Bill Aalbersberg
Paul Ralifo
Marika Tuiwawa

January, 2002

Antimicrobial Testing of Selected Fijian Plants, Part 2

by

Bill Aalbersberg, Paul Ralifo and Marika Tuiwawa

Institute of Applied Sciences,
The University of the South Pacific,
Suva, Fiji.

Abstract

Forty-six plants were extracted and tested for antimicrobial activity. These plants were collected in Viti Levu and some of them have been used traditionally as herbal remedies for certain illnesses. Seven microbes were used in the tests and include four bacteria, *Salinovibrio costicolla, Escherichia coli, Staphylococcus aureus, Bacillus subtilis,* and three fungi, *Candida albicans, Trychophyton mentagrophytes* and *Mirosporum gypseum.* The tests showed a variety of activities against these microorganisms.

The traditional use of these plants was also compared to the test results. Quite interestingly, all plants that were used for antibacterial or antifungal purposes showed this kind of activity in the laboratory tests.

1. Introduction

Every year rainforests are succumbing to deforestation and many plant species now face the danger of being overexploited. Hence, it is essential to know and appreciate the potential uses and values of local plants, in order to facilitate conservation of rain forests and their invaluable plant species. Many plants have a long history of traditional medicinal use and have been used extensively by traditional healers. Lately, modern scientists have borrowed ideas from traditional medicinal practices resulting in the development of new drugs.

In this study, the antimicrobial activities of forty six plant (Appendix 1) extracts were evaluated. These plants were collected in Viti Levu, Fiji and various parts of these plants were dried, ground and then extracted with methanol (MeOH). The extracts were then exposed to seven microorganisms, some of which are dermatophytes, and their activities recorded.

Four bacteria and three fungi were used in the assays. The four test bacteria included a marine Gram-negative bacterium, *Salinovibrio costicolla*, and three terrestrial bacteria, *Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis. E. coli* is Gramnegative and is known to be part of the flora of microorganisms that inhabit the guts of humans and animals. This bacterium can produce enterotoxins causing diarrhea in animals and man. *S. aureus* is a Gram-positive bacterium and is a common inhabitant of skin and nasal passages and can cause staphylococcal food poisoning, boils, meningitis, impetigo and pneumonia. *B. subtilis* is a Gram positive bacterium and is sometimes an opportunistic pathogen causing food poisoning. The three fungi include the yeast *Candida albicans, Trychophyton mentagrophytes* and *Microsporum gypseum. C. albicans* is a yeast that causes infections of the mucosal membranes of the body known as candidiasis. This fungus can grow in many forms such as unicellular, filamentous and as chlamydospores depending on the medium it is grown in. *T. mentagrophytes* (var. *mentagrophytesi*) and *M. gypseum* are common human dermatophytes causing cutaneous mycoses such as tinea pedis (atheletes foot), tinea corporis (ringworm of the smooth or bare parts of the skin), tinea cruris (ringworm of the groin), and tinea unguium (infection of the nail bed). Due to the pathogenic nature of these microorganisms these tests were done in a biosafety cabinet (class II) using sterile techniques.

2. Methodology

1. Sample preparations:

   All the samples were dried, ground and soaked in methanol overnight. The methanol (MeOH) extracts of the samples were then filtered and reduced under vacuum. This extraction process was repeated twice.

   The dried crude extracts (100mg) were then dissolved in 80% dimethyl sulfoxide (DMSO) in water to make concentrations of $250 mg.ml^{-1}$. The dissolved samples (10µL) were then transferred to sterile paper disks (6mm diameter) and dried and then used in the assays.

2. Antibacterial assay:

   This assay employed four bacteria, *S. costicolla, E. coli, B. subtilis* and *S. aureus*. Seed cultures (3mL) were prepared for each bacterium in the following way. Nutrient broth (NB) was used to culture *E. coli, B. subtilis* and *S. aureus*, however, marine broth (MB) was used for *S. costicolla*. The prepared medium (3mL) for each bacterium was autoclaved, cooled and inoculated with each bacterium using sterile 10µL loops and the cultures incubated at 30°C overnight while shaking.

   For the assay plates, nutrient agar (NA) was used to culture *E. coli, B. subtilis* and *S. aureus*, however, marine agar (MA) was used for *S. costicolla*. The media (50mL) for each bacterium was autoclaved and then cooled to 45°C and inoculated with overnight seed cultures. MA (50mL) was inoculated with 1.5mL of overnight broth culture of *S. costicolla,* while for the other test bacteria, 50mL of NA was inoculated with 50µL of overnight broth culture. The inoculated media were then poured into tissue culture plates, allowed to solidify, and then used in the bioassay.

Prepared sample disks were then placed on the assay plates together with a known standard for that particular bacterium and a positive control. The plates were incubated as follows, *E. coli* and *B. subtilis* plates were incubated at 37°C while *S. aureus* and *S. costicolla* were incubated at 30°C. The results were checked and recorded after 24 hours of incubation by measuring the diameter of the zone of inhibited microbial growth (mm). Corrected results were then taken by subtracting the diameter of the sample disks from the diameter of the zone of inhibition.

3. Anti-*Candida* assay:

Freshly prepared and autoclaved tryptic soy broth (TSB) was inoculated with *C. albicans* and incubated at 35 to 38°C, stationary overnight. After incubation, the seed culture was used to prepare a 10^{-1}dilution (10mL) overnight culture with an optical density between 0.05 and 0.5 using a spectrophotometer (A_{600}). The diluted seed culture (500μL) was then used to inoculate 125mL of molten Sabourad dextrose agar (SDA) at ~45°C. The inoculated media was then gently mixed and then poured into sterile petri dishes.

The plates were then allowed to dry and the sample disks were placed on the surface of the inoculated agar. A disk containing nystatin was assayed as a standard and a positive control was also assayed which contains only the solvent.

4. Antifungal assay:

Potato dextrose agar (PDA) was autoclaved and poured into sterile petri dishes and allowed to set and dry at room temperature. The plates were inoculated with thawed glycerolised fungal colonies. The inoculated plates were then incubated at 36-38°C for 7 days.

After incubation, a sterile inoculating needle was used to remove spores from the 7-day fungal colonies and on newly prepared PDA plates, the spore-loaded needle was dipped at three points. The inoculated plates were then incubated at 36-38°C for 7 days.

To inoculate the assay plates, mycelium plugs from the 7-day old 3-point inoculum plates were used. A sterile core bore (size 2) was used to punch into the agar at points covered with mycelium on the 3-point inoculum plates. The mycelium plugs were then carefully lifted using sterile forceps and placed upside down (the mycelium side of the plugs were placed faced down) on new PDA plates. The plates were then sealed and incubated (right side up) at 36-38°C for 4 days.

In the assay, a sample disk was loaded onto the 4-day assay plate at 1.5cm from the mycelium plug. A blank disk loaded with the solvent alone was placed (1.5cm from the mycelium plug) on the opposite side of the mycelium plug on the same plate as a positive control. The assay plates were then incubated (right side up) at 36 to 38°C for another 4 days. A standard prepared from 9mg of griseofulvin in 1mL of DMSO was also assayed for comparison purposes. Positive results were interpreted as having an inhibition zone between the mycelium growth and the sample disk as compared to the blank. All positive results were replicated and the average of the diameters of the zones of inhibited microbial growth was taken.

3. Results and discussion

All inhibition zones of less than 1mm were recorded as negative. In each of the assays, a standard was included to ensure that the test system was functioning properly and to compare the activity of the sample of interests with the standard. In addition, a positive control, which includes the solvent alone, was included, to measure the effect, if any, of the solvent in which the samples were dissolved.

The results from the microbial assays are listed in Table 1. The results of the controls are listed in Table 2.

Approximately half of the extracts showed activity against one of the bacteria and about a third of them against one of the fungi. Only the *Endospermum macrophyllum* wood extract was active against of yeast C*andida albicans.* This extract was also active against with three of the bacteria with large zones of inhibition and both fungi.

Interestingly, the most extensive compilation of medicinal activity of Fijian plants (Cambie and Ash, 1994) which summarises all written reports of such activity, makes no mention of this plant. Activity is reported for 450 of the roughly 2,500 plants found in Fiji. Of course, many traditional treatments have not been documented in writing and some of the most important ones may be family secrets.

The use of plants can also depend on availability. Many rare plants in Fiji are not used and some not even named in the local language due to this rareness.

Of the forty-six plants tested eighteen are not listed in Cambie and Ash. Seventeen are listed but the conditions for which they are reportedly used are not related to anti-bacterial or antifungal activity. Nine plants were used for anti-bacterial purposes, *Barringtonia asiatica, Vitex trifolia* var. subtrisecta, *Epipremnum pinnatum, Premna taitensis, Syzygium malaccense, Solanum torvum, Mussaenda raiateensis, Cordyline terminalis* and *Dillenia biflora.* All of these tested positive for antimicrobial activity, and all but *Barringtonia asiatica* for antibacteria activity. The leaves of two plants, *Heritiera littoralis* and *Xylocarpus granatum*, are used to treat thrush, a yeast intection of the tongue and both displayed strong antifungal activity.

Overall only 44% of plants that did not appear in Cambie and Ash showed any activity. For plants that were listed as used medicinally but not for antimicrobial uses 53% showed activity. As mentioned earlier, 100% of the 11 plants that were reported to be used extensively for antimicrobial purposes were also active in the laboratory. These results indicate a good correlation between traditional use by Fijians and activity tests in the laboratory.

One plant tested, *Homalanthus nutans*, has received considerable interest overseas as it contains prostratin, a potential anti-AIDS medicine. The extract from this plant also showed antimicrobial activity.

4. References

- Bergey's manual of systematic bacteriology, *Vol 1,* Kreig, N. R. and Hol, J. G. (eds), Williams and Wilkins, Baltimore, 1984.

- Bergey's manual of systematic bacteriology, *Vol 2,* Sneath, P. H. A.; Mair, N. S.; Sharpe, M. E. and Holt, J. G. (eds.), Williams and Wilkins, Baltimore, 1986.

- Cambie, R. C. and Ash, J. (1994) Fijian medicinal plants, CSIRO, Australia, 365pp.

- Medicinal plants in the South Pacific, WHO Regional Publications Western Pacific Series No. 19, 1998, 254pp.

TABLE 1: RESULTS OF THE BIOACTIVITY TESTS FOR THE PLANT EXTRACTS

Plant Code	Antimicrobial Activity (Zone of inhibition measured in mm).						
	B. subtilus	*E. coli*	*S. aureus*	*S. costicola*	*C. albicans*	*T. mentagrop hytes*	*M. gyseum*
3D						2	3
6D							2
13 D & T	10						
14D						2	3.5
15 D & T	9					2	2
16T	9					2	4
17 D & T						2	3
18D							
24D		7.5					
24T		8					
26D		8.5					
28D							
29D							
32D						3	2
33D		8					2
34D							
34T							
36T							
38T						3	4
41 D & T							
42D		10					
44D						3.5	
45							
45T							
47D							
50D	12						
51D	8						
36D	8						
53D							
57D							
54D							
57T	8					2	
58T	9		10				
58D							
61T							
62D	9						
62T							
63D	11		12				
73T							
76D							
79D	9						
80T	17		11		9	2	1.5
84D							
87D							
92D			10				
95T	9						
96T							
102D							
105D				8			
106D	9		11			2	
108D						2	
109D	9		9			2	
110D	9		11			2	

TABLE 2: AVERAGE ZONES OF INHIBITION FOR THE RESPECTIVE CONTROLS

Controls	Measured in mm						
	B. subtilus	*E. coli*	*S. aureus*	*S. costicola*	*C. albicans*	*T. mentagrophytes*	*M. gyseum*
Penicillin G	30	-	39	-	-	-	-
Polymixcin B	-	22	-	25	-	-	-
Nystatin	-	-	-	-	10	-	-
Griseofulvin	-	-	-	-	-	6	8

Appendix 1. List of Plants Tested in the Assays

Sample Code	Scientific Name	Plant Part
3	*Barringtonia asiatica*	leaves
6	*Heritiera littoralis*	leaves
13	*Vitex trifolia* var *subtrisecta*	bark
14	*Aleurites moluccana*	leaves
15	*Epipremnum pinnatum*	bark
16	*Homalanthus nutans*	wood
17	*Bambusa vulgaris*	leaves
18	*Cerbera manghas*	leaves
24	*Premna taitensis*	leaves, wood
26	*Pandanus caricosus* (Whitmeeanus)	leaves
28	*Syzygium malaccense*	leaves
29	*Parasponia andersonii*	leaves
32	*Xylocarpus granatum*	leaves
33	*Myristica grandifolia*	leaves
34	*Lumnitzera littorea*	leaves, wood
36	*Syzygium richii*	wood
38	*Maesa tabacifolia*	wood
41	*Passiflora foetida* var. *hispida*	wood
42	*Physalis peruviana*	leaves
44	*Hernandia peltata*	leaves
45	*Tarenna sambucina*	wood
47	*Coccinea grandis*	leaves
50	*Alphitonia zizyphoides*	leaves
51	*Solanum torvum*	leaves
53	*Ervatamia orientalis*	leaves
54	*Gironniera celtidifolia*	leaves
57	*Mussaenda raiateensis*	leaves, wood
58	*Arytera brackenridgei*	leaves, wood
61	*Fagraea berteroana*	wood
62	*Cordyline terminalis*	leaves, wood
63	*Podocarpus affinis*	leaves
73	*Dillenia biflora*	wood
76	*Ficus theophrastoides*	leaves
79	*Pittosporum rhytidocarpum*	leaves
80	*Endospermum macrophyllum*	wood
84	*Palaquium vitilevuense*	leaves
87	*Palaquium porphyreum*	leaves
92	*Codiaeum variegatum*	leaves
95	*Adenanthera pavonina*	wood
96	*Trichospermum richii*	wood
102	*Merremia peltata*	leaves
105	*Geissois ternata*	leaves

106	*Garcinia myrtifolia*	leaves
108	*Endiandra gillespiei*	leaves
109	*Turrillia vitiensis*	leaves
110	*Smilax vitiensis*	leaves