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Abstract
Sixteen 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 *Salinovibrio costicolla, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Candida albicans Trychophyton mentagrophytes* and *Mirosporum gypseum*. The tests showed a variety of activities against these microorganisms.

1. Introduction

Every year rainforests succumb to deforestation and plant species become extinct. 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. Lately, modern scientists have borrowed ideas from traditional medicinal practices resulting in the development of new drugs.

In this study, the antimicrobial activities of sixteen plant (**Table 1**) extracts were investigated. These plants were collected in Viti Levu and various parts of these plants were dried, ground and then extracted with methanol. The extracts were then exposed to seven microorganisms and their activities recorded. Some of the plants collected were used traditionally as herbal remedies and their uses are listed in **Table 2**.

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 Gram negative and is known to be part of the flora of microoganisms 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

Table 1. List of plants tested in the assays.

Sample code	Scientific name	Part of plant used
174D	*Crossostylis harvei*	Leaves
173	Flame ginger *	Whole
153	*Dolicholobium macgregori*	Leaves and flowers
157T	*Crossostylis seemanii*	Trunk
162	*Costus speciousus*	Stem and leaves
159	*Heliconia paka*	Leaves and stalk
Dilo seed	*Calophyllum inophyllum*	Seeds
169D	*Neuburgia alata*	Leaves
153T	*Dolicholobium macgregori*	Trunk
155T	*Pittosporum pickeringii*	Trunk
46D	*Mikania micrantha*	Whole
151	*Pittosporum rhytidocarpum*	Trunk and leaves
167	*Calanthe sp.*	Whole
170	*Spathoglottis pacifica*	Whole
169	*Neuburgia corynocarpa*	Whole
174T	*Agathis macrophyllum*	Trunk

* The scientific name of this plant is not known however has been identified by its common name of flame ginger.

Table 2. Traditional medicinal uses of plants in Fiji.

Sample code	Traditional medicinal use
157T	Used to treat headaches, occurrence of blood in the urine, unconsciousness and dysentery. A combination of the bark of this plant and other plants is used to treat fractures.
Dilo seed	Oil from the seed is used to treat rheumatism, wounds and coral sores and bruises. The leaf is used to treat eye irritations and conjunctivitis. The plant is also used to treat toothache and tuberculosis.
155T	The plant is used to treat constipation, cuts, wounds and stomach trouble.
46D	The leaves are used to treat skin irritations, bees and wasp stings, stop bleeding and boils of the armpit. It is also used for stomach aches, high blood pressure and diabetes, cancer and hastens birth for expectant mothers.
151	The plant is used by mothers after childbirth.
170	Used to treat pain in the joints.
169	The roots and leaves are used together with *Mikania micrantha* to treat backache, headache and vaginal bleeding.

opportunistic pathogen causing food poisoning. The three fungi include the yeast *Candida albicans* and two moulds *Trychophyton mentagrophytes* and *Microsporum gypseum. C. albicans* causes infections of the mucosal membranes of the body known as candidiasis. *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 microoganisms 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. This extraction process was repeated twice. The combined methanol extracts of the samples were then filtered and reduced under vacuum.

   The dried crude extracts (100mg) were then dissolved in 80% dimethyl sulfoxide (DMSO) in water to make concentrations of 250mg.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 media (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 used as 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 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 disk was also assayed which contained only the solvent.

4. *Anti-fungal 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 face 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. 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

Table 3. Results of the bioactivity tests for the plant extracts:

Plant (part of plant used)	Antimicrobial activity (Ave. zone of inhibition (mm))						
	S. costicolla	*E.coli*	*S. aureus*	*B. subtilis*	*Candida*	*T. mentagrophytes*	*M. gypseum*
Crossostylis harvei (leaves)	-	-	3.0	3.0	-	-	-
Flame ginger (whole)	2.0	-	3.0	4.0	-	3.5	-
Dolicholobium macgregori (leaves and flowers)	-	-	-	-	-	-	-
Crossostylis seemanii (trunk)	-	-	-	-	-	-	-
Costus speciosus (stem and leaves)	-	-	-	-	-	1.5	1.5
Heliconia paka (leaves and stalk)	-	-	-	-	-	-	-
Calophyllum inophyllum (seeds)	3.0	-	-	-	-	-	-
Neuburgia alata (leaves)	-	-	-	-	-	-	1.0
Dolicholobium macgregori (trunk)	-	-	-	-	-	2.0	1.0
Pittosporum pickeringii (trunk)	-	-	3.0	-	-	-	-
Mikania micrantha (whole)	12.5	-	8.0	5.0	-	-	2.0
Pittosporum rhytidocarpum (trunk and leaves)	-	-	-	-	-	-	2.0
Calanthe sp. (whole)	-	-	-	-	-	-	-
Spathoglottis pacifica (whole)	-	-	-	-	-	-	-
Neuburgia corynocarpa (whole)	-	-	-	-	-	1.0	1.5
Agathis macrophyllum (trunk)	-	-	-	-	-	-	-
Ampicillin	9.0	-	NA	NA	NA	NA	NA
Penicillin-G	NA	13.0	30.0	19.0	NA	NA	NA
Nystatin	NA	-	NA	NA	3.0	NA	NA
Griseofulvin	NA	-	NA	NA	NA	6.5	9.0

All inhibition zones of less than 1mm were recorded as negative. "NA" in the above table means not applicable. 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 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 showed that some of these plant extracts showed variable antimicrobial activity. None of the extracts however showed any activity against *C. albicans*. The strongest antimicrobial activity was recorded for *Mikania micrantha* against *S. costicolla* (even more active than ampicillin) and it showed activity against *S. aureus, B. subtilis* and *M. gypseum*. This plant is used traditionally in Fiji to treat skin irritations, insect stings, boils of the armpits and is known to exhibit antimicrobial activity. *Calophyllum inophyllum* is known to exhibit antibacterial activity and the leaves of *Pittosporum rhytidocarpum* are known to produce antimicrobial compounds. However in the assay, *Calophyllum inophyllum* showed only low activity (3.0mm) against *S. costicolla* and no activity against the other microbes for that concentration. *Pittosporum rhytidocarpum* also exhibited moderate activity against *M. gypseum* and no activity against the other microbes for that concentration.

Not all the plants studied are used traditionally as herbal remedies. However, these types of studies can identify potential uses of various plant species which can be of commercial value.

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.