



Article

Antimicrobial Properties against Human Pathogens of Medicinal Plants from New Zealand

Syahidi Safian ¹, Hafiz Majid ^{1,2}, Simon Swift ^{3,*} and Filipa V. M. Silva ^{4,*}

- ¹ Chemical and Materials Engineering Department, Faculty of Engineering, Waipapa Taumata Rau University of Auckland, Private Bag 92019, Auckland 1142, New Zealand; msaf458@aucklanduni.ac.nz (S.S.); hafizam@ums.edu.my (H.M.)
- ² Seaweed Research Unit, Faculty of Science and Natural Resources, Universiti Malaysia Sabah, Kota Kinabalu 88400, Sabah, Malaysia
- ³ Molecular Medicine and Pathology, Faculty of Medical and Health Sciences, Waipapa Taumata Rau University of Auckland, Private Bag 92019, Auckland 1142, New Zealand
- ⁴ LEAF, Associate Laboratory Terra, Instituto Superior de Agronomia (ISA), Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal
- * Correspondence: s.swift@auckland.ac.nz (S.S.); fvsilva@isa.ulisboa.pt (F.V.M.S.)

Abstract: The emergence of resistant microorganisms towards standard antibiotics has stimulated an on-going exploration for new sources of antimicrobials. The microbial susceptibility of extracts produced from leaf, bark, or rhizome parts of nine different New Zealand bushes was investigated using liquid broth dilution and agar plating techniques. Minimum inhibitory (MIC) and lethal concentrations (MLC) were expressed in micrograms of dry extract per milliliters of solution. The lowest MIC of 62.5 µg/mL was determined for methanol extract of *Kunzea ericoides* against *Bacillus cereus* and *Candida albicans*, and ethyl acetate extract of *Pseudowintera colorata* against *Staphylococcus aureus*. Additionally, *K. ericoides* also presented the lowest MLC of 250 µg/mL against *S. aureus* and *B. cereus* (methanol extract), and against *S. aureus* (ethyl acetate extract). The methanol extract of *Weinmannia racemosa* was lethal to *B. cereus* (MLC = 250 µg/mL). Some of the extracts of *Phormium tenax*, *Schefflera digitata*, and *Pomaderris kumeraho* were antimicrobial against *S. aureus* and *B. cereus* (MIC = 500 µg/mL). The extracts of *Geniostoma ligustrifolium* and *Meliclytus ramiflorus* plants did not exhibit antimicrobial activity.

Keywords: shrub; kānuka; horopito; kāmahī; antibiotic; microbial susceptibility; minimum inhibitory concentration; minimum lethal concentration; MIC; MLC



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1. Introduction

Hospital-acquired infections and the increasing number of drug-resistant pathogens are major problems in hospitals [1,2]. Over the past few years, health agencies have been trying to minimize the use of antibiotics through various advertising campaigns and to sensitize people towards public health awareness. Despite the development of antibiotics, bacterial and fungal infections are still a public health concern, and the continued emergence of numerous drug-resistant pathogen strains poses a challenge. Some microorganisms are particularly problematical, and this has led the WHO to publish a list of 12 priority organisms for which new antibiotics are urgently needed in 2017 [3]. Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) are a major concern in hospitals as they become resistant to the frequently used antibiotics. In another study, it has been reported that strains such as *Staphylococcus aureus* SA1199 B (NorA) and XU212 (TetK, PBP2a) are highly resistant to antibiotics. Overexpression of efflux pumps such as NorA is a common mechanism of resistance found in multi-drug-resistant *S. aureus* due to the mechanism of efflux [4]. Today, bacterial resistance is recognized as a threat to human health. The problem is especially serious in more susceptible patients during chemotherapy and after organ transplantation because of immunosuppression.

The emergence of multi-drug-resistant (MDR) strains in the hospital or other health care facilities has resulted in the emergence and re-emergence of difficult to treat nosocomial infections in patients [5,6]. This has contributed to higher morbidity and mortality rates, increased health care costs due to treatment failures, and longer hospital stays [6,7]. Multi-drug resistant microorganisms can cause fatal infection in hospitals and nursing homes, where patients with open wounds, invasive devices, and immunodeficiency are at higher risk of infection than healthy people. Therefore, the continuing spread of multi-drug-resistant strains and the increased abuse of antibiotics represent a main challenge in present days [6,8].

As a consequence, researchers explore new approaches such as substances derived from natural products to replace, or to be used in combination with conventional antibiotics, on the basis that these natural substances might have a different mode of action and would have potential to combat the resistant microbial strains [9,10]. Plant materials are an interesting source of natural products with potential antimicrobial properties [11]. There has been an encouraging interest towards more detailed studies of plant resources as natural antibiotic substitutes due to their availability and fewer side effects (e.g., toxicity), as well as better biodegradability as compared with the available antibiotics. Plant extracts are known to be active against a wide variety of microorganisms, including the Gram-negative and Gram-positive bacteria in which Gram-negative bacteria were shown to be generally more resistant than Gram-positive.

In New Zealand, there are a number of endemic plants used locally as medicines, for aromatherapy and indirectly in clinical medicine [12–17]. The development of novel molecules isolated from NZ endemic medicinal plants is supported by both mātauranga Māori and by western science [18]. Although New Zealand presents a rich and unique flora of native plants, only a few plants have been investigated for antimicrobial properties [19]. Table 1 shows the scientific, common names, and some of the medicinal properties of New Zealand bushes/small trees selected for this study. A study conducted by Wan et al. (1996) with 231 ethanol/aqueous extracts from New Zealand plants revealed inhibitory activity against HIV-1 protease by *K. ericoides*, *P. tenax*, *P. kumeraho*, *P. colorata*, *S. digitata*, and *W. racemosa* extracts, which may indicate the potential of these species also antimicrobials [20]. In this study, 18 extracts from nine New Zealand shrubs were produced, and the antimicrobial potential against five pathogen strains commonly used for microbial susceptibility testing was investigated.

Table 1. Selection of New Zealand trees and shrubs investigated in this study: Scientific names, common Māori names, and ethnopharmacological uses *.

Scientific Name	Local Name	Type of Plant	Ethnopharmacological Uses	Scientist
<i>Kunzea ericoides</i>	kānuka	tree	The essential oils of several species of <i>Myrtaceae</i> have been found to have activity against <i>Staphylococcus aureus</i> ; it was also mentioned in the discussion that they support antimicrobial results against <i>S. aureus</i> . Bark decoction relieved diarrhea and dysentery. A weak infusion of the leaves was often given to a patient with a high temperature	Richard A, 1832
<i>Pseudowintera colorata</i>	horopito, pepper tree	small tree	Leaves were used to cure skin disease, venereal diseases, diarrhea; decoction of the leaves was used for stomachache; leaves were chewed for toothache (dental analgesic); leaves rubbed on breast when weaning infants. Sesquiterpenoid dialdehyde polygodial has strong antibiotic activity against <i>Candida albicans</i> (support the antimicrobial result against <i>Candida</i>)	Raoul E, 1844

Table 1. Cont.

Scientific Name	Local Name	Type of Plant	Ethnopharmacological Uses	Scientist
<i>Weinmannia racemosa</i>	kāmahi	tree	The inner bark is laxative, and the bark infused in boiling water was a tonic medicine	Linnaeus C. von filius, 1782
<i>Phormium tenax</i>	harakeke, flax	evergreen perennial plant	The blanched base of the leaf or root was beaten to pulp, heated, or roasted, and applied hot to bring forward abscesses or tumors; leaves and roots have beneficial effects for cutaneous diseases such as ringworm, or venereal diseases.	Forster JR and Forster G, 1776
<i>Schefflera digitata</i>	patete, umbrella tree, seven finger	tree	The sap was used on scrofulous sores and ringworm; the leaves contain farcarindiol, an acetylenic alcohol which shows remarkable specific activity against common dermatophyte fungi such as those causing ringworm.	Forster JR and Forster G, 1776
<i>Pomaderris kumeraho</i>	kumarahou	shrub	Liquid from boiled leaves is a well-known relief for all chest complaints, bronchitis, and pulmonary tuberculosis; ointment applied for skin cancer; beneficial effect on kidneys	Cunningham A, 1839
<i>Hebe stricta</i>	koromiko	herb	Leaves infusion is a powerful astringent for dysentery and other complaints; the leaves were chewed as a remedy for diarrhea; decoction taken for ulcers and venereal diseases. Top shoots and young leaves (green or dried) boiled or eaten raw were a remedy for kidney and bladder troubles.	Jussieu AL de, 1789
<i>Geniostoma ligustrifolium</i>	hangehange	shrub	The sap was applied to skin diseases of children.	Cunningham A, 1838
<i>Melicytus ramiflorus</i>	māhoe	small tree	The leaves were boiled, and the liquid was used to bathe parts affected by rheumatism. The boiled leaves were bandaged on surfaces affected with scabies and a plaster of steamed leaves was placed over a stomach wound.	Forster JR and Forster G, 1776

* Information extracted from: Māori Plant Use, <https://maoriplantuse.landcareresearch.co.nz> (accessed on 16 May 2022); Brooker et al., 1987 [19]; Riley and Enting, 1994 [21].

2. Materials and Methods

2.1. Plant Species

The following species/part of the plant were used in this study: kānuka *Kunzea ericoides* (leaves), horopito *Pseudowintera colorata* (leaves), kāmahi *Weinmannia racemosa* (bark), harakeke *Phormium tenax* (rhizomes), patē *Schefflera digitata* (leaves), kumarahou *Pomaderris kumeraho* (leaves), koromiko *Hebe stricta* (leaves), hangehange *Geniostoma ligustrifolium* (leaves), and māhoe *Melicytus ramiflorus* (leaves) (Table 1). The plants were chosen based on past publications, local and traditional uses, medicinal properties, availability of the plant, and ease of extraction.

Plant materials harvested during late Spring/Summer were supplied and identified by Fred Allen, an expert with a profound knowledge of New Zealand native plants/trees/bushes, from Kiwi Plants Ltd. (Member of Te Paepae Matua mo Te Rongoa, Nga Ringa Whakahaere o te iwi Māori, and Natural Products New Zealand). The plant samples, purchased as dried flakes at moisture content lower than 5%, were further dried in the dark inside an oven at 30–40 °C for 48 h to remove possible moisture. Then, the dried materials were ground, vacuum-sealed, and stored at room temperature under dark conditions until extraction.

2.2. Preparation of Plant Extracts Using Soxhlet Method

For each plant, ten grams of finely ground sample was extracted with 125 mL of either methanol or ethyl acetate using a Soxhlet extractor (Extraction System B-811, BÜCHI Labortechnik AG, Flawil, Switzerland). The solvents ethyl acetate, with relatively low polarity, and methanol, with high polarity, were chosen to ensure the extraction of all sorts of compounds, depending on their polarity and affinity with each solvent. Based on preliminary work, the optimized period for extraction of 10 g ground sample is 12 cycles (40–60 min). The ideal extraction temperature for each solvent was set according to their boiling points. The solution was filtered and concentrated by using a rotary evaporator (Rotavapor R-215, BÜCHI Labortechnik AG, Flawil, Switzerland). The concentrated extracts were further evaporated to dryness via air-drying at ambient temperature for 24 h. All residues were stored in the dark at $-70\text{ }^{\circ}\text{C}$ prior to analysis. In general, the methanol extracts were sticky solids with different colors (brown, reddish, dark green, greenish) and the ethyl acetate extracts were either dry or sticky solids (green, black, brown).

2.3. Preparation of Extracts Suspensions for Antimicrobial Testing

The plant extracts were taken out from the $-80\text{ }^{\circ}\text{C}$ freezer and thawed at room temperature. Based on the solubility of the dried extracts produced in DMSO, 0.04 g (for *S. digitata*, *K. ericoides*, *H. stricta*, *P. tenax*, *W. racemosa*, *G. ligustrifolium*) or 0.02 g (for *P. colorata*, *P. kumeraho*, *M. ramiflorus*) of the plant extract was weighed and added into 1 mL of dimethyl sulfoxide (DMSO) in a tube to produce extracts with concentrations of 40,000 and 20,000 $\mu\text{g}/\text{mL}$, respectively. The suspension was mixed well until homogeneity was reached and kept in the $-80\text{ }^{\circ}\text{C}$ freezer.

2.4. Microorganisms

Bacterial and fungal species representative of key pathogens and widely accepted as testing strains for antimicrobial activity were chosen. Two pathogenic Gram-positive bacteria, *Staphylococcus aureus* (ATCC 6538) and *Bacillus cereus* (ATCC 10702); one pathogenic Gram-negative bacterium, *Escherichia coli* (ATCC 25922); a pathogenic yeast, *Candida albicans* (ATCC 10231); and a mould, *Aspergillus niger* (ATCC 16404) (recently renamed to *Aspergillus brasiliensis*) were used. The microorganisms were obtained from the New Zealand Reference Culture Collection, Institute of Environmental Science and Research (ESR, Wellington, New Zealand).

2.5. Preparation of Agar and Broth Media, and Microbial Cultures

2.5.1. Preparation of Agar Plates and Broth Growth Media

For the preparation of different types of agar plates, first 7.5 g of granulated agar was added to 500 mL of distilled water. Then, for Tryptic Soy Agar (TSA) 15 g of Tryptic Soy broth powder was added, while for Mueller Hinton (MH) II cation-adjusted agar, 11 g of MH II cation-adjusted broth powder was added. For Yeast Extract–Peptone–Dextrose (YPD), agar 25 g of YPD broth powder was added. Then each solution was sterilized by autoclaving, and plates were filled with the warm solution and left to solidify, then inverted. To prepare YPD broth medium, 25 g of YPD broth was added to 500 mL of distilled water, while for MH II cation-adjusted broth medium 11 g of MH II cation-adjusted broth powder was mixed with 500 mL of distilled water.

2.5.2. Preparation of Microorganism Colonies in Agar Plates

All the microorganisms except *A. niger* were streaked on TSA agar plates and incubated overnight at $37\text{ }^{\circ}\text{C}$. The plates with microbial colonies were then stored in a refrigerator at $4\text{ }^{\circ}\text{C}$ for further use throughout the experiments when needed. For *Aspergillus niger*, the streaking was carried out on a YPD agar plate and kept at $28\text{ }^{\circ}\text{C}$ until a fair amount of spore growth had been achieved (judged based on the intensity of the black color of the spores), which took approximately two weeks.

2.5.3. Preparation of Fresh Overnight Microorganism Cultures in Broth and Inoculum

The following procedure was used for *E. coli*, *S. aureus*, *B. cereus*, and *C. albicans*. For each species, a small amount of the microorganism colony was streaked out from the agar plate using a sterile plastic loop and mixed into 10 mL of Mueller Hinton II cation-adjusted broth in a falcon tube (50 mL, V-bottom). The cultures were mixed well and incubated overnight in a rotary incubator at 37 °C and 200 rpm. For antimicrobial testing, 2 µL (for *E. coli*), 20 µL (for *S. aureus*), 40 µL (for *C. albicans*), and 12 µL (for *B. cereus*) were taken out from the respective overnight cultures and each was added into 20 mL of MH II cation-adjusted broth in different falcon tubes to yield approximately a final concentration of the microorganism of 1×10^6 cfu/mL. The inoculum was mixed well in the falcon tubes.

2.5.4. Preparation of *A. niger* Inoculum

For the preparation of *A. niger* inoculum, firstly, about 10–15 mL of YPD broth was poured onto the agar plate containing grown colonies. Then, the agar surface was streaked by using an L-shaped stick to harvest the spores. After that, all the harvested spores were poured into the falcon tube. A series of half dilution was performed by adding 1 mL of YPD broth with 1 mL of the harvested spores inside a yellow-capped tube. After a good vortex, 1 mL was taken out from this mixture and added into the second tube labeled '2' containing 1 mL of YPD broth. The steps were continued 4–5 times to reach 4–5 half dilutions. The correct dilution for the assay was chosen by counting the spores using a hemocytometer.

2.6. Determination of MIC and MLC of Plants Extracts

The antimicrobial activity of each extract was quantified by determining the minimum inhibitory concentration (MIC) using the liquid broth micro-dilution and minimum lethal concentration (MLC) using the agar plating technique from the NCCLS standards [22]. For bacteria and *Candida albicans*, MH broth and agar were used, while for the mould *A. niger*, YPD was selected as it provides a consistent result with this particular mould.

2.6.1. Determination of MIC

The plant extract suspension was thawed at room temperature and vortexed. A microplate was prepared for each microorganism (*S. aureus*, *B. cereus*, *E. coli*, *C. albicans*) as follows: 10 µL of plant extract suspension and 90 µL of MH II cation-adjusted broth were added into 3 wells of the first row (3 replicates). Then a blank sample containing only DMSO (no plant extract) was also tested for any antimicrobial activity by adding 10 µL of DMSO and 90 µL of MH II cation adjusted broth into another 3 wells (3 replicates). Next, 50 µL of Mueller Hinton II cation-adjusted broth were added into each well, starting from the second row until the eighth row. Two-fold serial dilution was performed by taking 50 µL out from the first row into the wells of second row. The dilution was performed just until the wells of seventh row. Then, the last 50 µL from the wells of seventh row was discarded, resulting in each well containing 50 µL volume and the wells of the eighth row being the extract-free control (not containing plant extract suspension). Lastly, 50 µL of 10^6 CFU/mL microorganism inoculum was added into each well and mixed properly. For *A. niger* mould, the same procedure was followed but using YPD broth.

As a result, the plant extract suspension with 40,000 µg/mL was diluted from an initial concentration of 2000 µg/mL to a final concentration of 31.25 µg/mL, while the plant extract suspension with 20,000 µg/mL was diluted from an initial concentration of 1000 µg/mL to a final concentration of 15.63 µg/mL. Three replicates were made for each microbe/extract test. The microtiter plate was then covered and left for overnight incubation at 37 °C for the bacteria and yeast, and incubation for 2 days at 28 °C for *Aspergillus niger*, with 200 rpm. The shaking was needed to help the extract remains in suspension over the incubation period. The lowest concentration of the extract that will result in a complete inhibition of visible growth in the broth when compared to the extract free control will be the MIC.

2.6.2. Determination of MLC

Each MH II cation-adjusted agar plate was divided into 8 segments and labeled based on the respective concentration: 2000, 1000, 500, 250, 125, 62.5, 31.25, and 0 µg/mL for 40,000 µg/mL extract suspension or 1000, 500, 250, 125, 62.5, 31.25, 15.63, and 0 µg/mL for 20,000 µg/mL extract suspension. Twenty µL of sample was pipetted aseptically from each well and plated onto MH II cation-adjusted agar on its respective concentration section. The plates were allowed to air-dry, and the plates were then inverted and incubated at 37 °C for 18–24 h. MLC was evaluated as the lowest extract concentration at which no growth was observed on the plates. For *A. niger* the same procedure was used except the agar medium which was replaced by YPD and the incubation temperature was 28 °C.

2.7. Statistical Analysis of Data

Although the experiments were repeated three times for each plant extract against each microorganism, the average and standard deviation are not appropriate for MIC and MLC concentrations. The active concentrations of the extracts are exact amounts against each microorganism, and thus cannot be averaged. Instead, the median values were determined using GraphPad Prism software.

3. Results and Discussion

Although aromatic and medicinal plants have been used from ancient times as natural remedies and are considered as alternatives to complement the synthetic medications, in-depth scientific investigations must be conducted to study the antimicrobial properties of plant extracts. Thus, in this study, microbial susceptibility tests were conducted with 18 extracts produced from nine New Zealand endemic shrubs using liquid broth micro-dilution and plating techniques.

3.1. MIC and MLC of Antimicrobial Plant Extracts

Table 2 shows the MIC and MLC results for all the plant extracts tested. The results of *G. ligustrifolium* and *M. ramiflorus* are not in Table 2, as no antimicrobial activity was detected against the five microorganisms tested, so MLCs were not determined. *K. ericoides* (methanol extract) was the most antimicrobial, presenting the lowest MIC of 62.5 µg/mL against both *B. cereus* and *C. albicans* and 125 µg/mL against *S. aureus*. This extract showed a MLC of 250 µg/mL against both *S. aureus* and *B. cereus*. The ethyl acetate extract of the same plant was less antimicrobial, with a MIC of 125 µg/mL against both *S. aureus* and *B. cereus*, 250 µg/mL against *C. albicans*, and the MLC > 2000 µg/mL against *B. cereus*. The antimicrobial properties of *K. ericoides* against *S. aureus* are in agreement with a previous study [21]. Bloor (1995) also detected antimicrobial activity of *K. ericoides* aqueous methanolic extract against *S. aureus* and *C. albicans* [12]. As opposed to *K. ericoides*, the ethyl acetate *P. colorata* was much more active than the methanol extract, showing a MIC of 62.5 µg/mL against *S. aureus* compared with 1000 µg/mL for methanolic extract. A MIC of 125 µg/mL was shown for the methanol extract against *B. cereus* and *C. albicans*, as opposed to 500 µg/mL for the ethyl acetate extract of the same plant. With respect to *C. albicans*, ethyl acetate of *P. colorata* presented a MLC of 250, which was half of the MLC shown by the methanol extract. Previous results have also demonstrated good antimicrobial properties of *P. colorata* against *C. albicans* [23].

With respect to *W. racemosa*, methanol extract showed antimicrobial activity against *S. aureus* and *B. cereus* with the MIC of 250 µg/mL respectively and *C. albicans* with the MIC of 125 µg/mL. In addition, *W. racemosa* (methanol) also presented the MLC of 2000 µg/mL and 250 µg/mL against *S. aureus* and *B. cereus*, respectively. The ethyl acetate extract of *W. racemosa* displayed the MIC of 250 µg/mL against both *S. aureus* and *B. cereus* and 2000 µg/mL against *C. albicans*. *W. racemosa* (ethyl acetate) also had the MLC value of 2000 µg/mL against both *S. aureus* and *C. albicans*. Aqueous methanol extracts of *W. racemosa* were antimicrobial against *Pseudomonas aeruginosa*.

Table 2. Minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of methanol and ethyl acetate extracts of New Zealand medicinal plants *.

Plant Species	Extract	Concentration in µg/mL	Microorganisms				
			<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Candida albicans</i>	<i>Escherichia coli</i>	<i>Aspergillus niger</i>
<i>Kunzea ericoides</i>	Methanol	MIC	125	62.5	62.5	>2000	>2000
		MLC	250 ^a	250	>2000	>2000	>2000
	Ethyl acetate	MIC	125	125	250	>2000	>2000
		MLC	250	>2000	>2000 ^a	>2000	>2000
<i>Pseudowintera colorata</i>	Methanol	MIC	1000	500	500	>1000	>1000
		MLC	>1000 ^a	>1000	500	>1000	>1000
	Ethyl acetate	MIC	62.5	125	125	>1000	>1000
		MLC	>1000 ^c	>1000	250 ^a	>1000	>1000
<i>Weinmannia racemosa</i>	Methanol	MIC	250	250	125	>2000	>2000
		MLC	2000 ^b	250	>2000 ^a	>2000	>2000
	Ethyl acetate	MIC	250	250	2000	>2000	>2000
		MLC	2000 ^a	>2000 ^b	2000	>2000	>2000
<i>Phormium tenax</i>	Methanol	MIC	1000	2000	2000	>2000	>2000
		MLC	>2000 ^a	>2000	>2000	>2000	>2000
	Ethyl acetate	MIC	500	500	>2000	2000	>2000
		MLC	2000 ^a	>2000 ^a	>2000	2000	>2000
<i>Schefflera digitata</i>	Methanol	MIC	2000	500	2000	>2000	>2000
		MLC	>2000	>2000	>2000	>2000	>2000
	Ethyl acetate	MIC	1000	1000	1000	>2000	>2000
		MLC	1000 ^a	>2000	2000 ^a	>2000	>2000
<i>Pomaderris kumeraho</i>	Methanol	MIC	1000	500	1000	>1000	>1000
		MLC	>1000	>1000	>1000	>1000	>1000
	Ethyl acetate	MIC	>1000	>1000	>1000	>1000	>1000
		MLC	>1000	>1000	>1000	>1000	>1000
<i>Hebe stricta</i>	Methanol	MIC	>2000	2000	>2000	>2000	>2000
		MLC	>2000	>2000	>2000	>2000	>2000
	Ethyl acetate	MIC	nd	nd	nd	nd	nd
		MLC	nd	nd	nd	nd	nd

* *Pseudowintera colorata*, *Pomaderris kumeraho*, and *Meliclytus ramiflorus* extracts were not soluble at 2000 µg/mL, so the maximum concentration tested was 1000 µg/mL; no antimicrobial activity was detected for the methanol and ethyl acetate extracts of *Geniostoma ligustrifolium* (>2000 µg/mL), and *Meliclytus ramiflorus* (>1000 µg/mL) at the highest concentration tested, and the results are not presented in the table; nd = not determined due to its high value of MIC in all methanol extracts; ^a Presented partial lethality at one concentration lower; ^b Presented partial lethality at two concentrations lower; ^c Presented partial lethality at three concentrations lower.

In general, *P. tenax* (ethyl acetate) was more antimicrobial than *P. tenax* (methanol) because the former inhibited or/and killed microorganisms at lower concentrations. *P. tenax* (ethyl acetate) indicated an activity against *S. aureus* with the MIC of 500 µg/mL and MLC of 2000 µg/mL, *B. cereus* with the MIC of 500 µg/mL, and *E. coli* with both MIC and MLC of 2000 µg/mL. MIC of methanol extract of *P. tenax* against *S. aureus*, *B. cereus*, and *C. albicans* was 1000 µg/mL, 2000 µg/mL, and 2000 µg/mL respectively.

The ethyl acetate extract of *S. digitata* revealed activity against *S. aureus*, *B. cereus*, and *C. albicans* with a MIC of 1000 µg/mL. *S. digitata* (EA) also showed the MLC of 1000 µg/mL against *S. aureus* and 2000 µg/mL against *C. albicans*. The methanol extract of *S. digitata* presented MICs of 2000 µg/mL, 500 µg/mL, and 2000 µg/mL against *S. aureus*, *B. cereus*, and *C. albicans*, respectively.

The methanol extract of *P. kumeraho* was antimicrobial against *S. aureus*, *B. cereus*, and *C. albicans* with the MIC of 1000 µg/mL, 500 µg/mL, and 1000 µg/mL respectively. The ethyl acetate extract did not exhibit any antimicrobial activity the five microorganisms tested.

The data indicated that methanol extract of *H. stricta* showed activity against *B. cereus* with a MIC of 2000 µg/mL. No activity was detected against the other four microorganisms tested.

A screening study carried out with methanol extracts from New Zealand endemic plants also showed antibiotic activity of the same plants we tested [24]: *P. colorata* exhibited activity against *E. coli*, *S. aureus*, and *C. albicans*; *W. racemosa* showed activity against *E. coli* and *S. aureus*; and *Phormium tenax* extracts against *S. aureus*.

3.2. Nature of Antimicrobial Effect

To determine the nature of antimicrobial effect of plant extracts, the ratio MLC/MIC was used. This ratio was calculated only for the antimicrobial plant extracts presenting MIC and MLC values. If the ratio was lower than 4, the extract was classified as microbicidal, which means it has a lethal effect. If the ratio was greater than 4, the extract was classified as microbiostatic, which means it inhibits growth, but is not lethal [25,26]. Table 3 shows the results. The following plant extracts are microbicidal as they presented a MLC/MIC ratio ≤ 2 : *K. ericoides* methanol and ethyl acetate extracts against *S. aureus*; *P. colorata* methanol and ethyl acetate extracts are fungicidal against *C. albicans*; *W. racemosa* methanol extract is bactericidal against *B. cereus* and the ethyl acetate extract is fungicidal against *C. albicans*; *P. tenax* ethyl acetate extract is bactericidal against *E. coli*; *S. digitata* ethyl acetate extract is microbicidal against *S. aureus* and *C. albicans*.

Table 3. MLC/MIC ratio for antimicrobial plant extracts against susceptible microorganisms *.

Plant Common Name	Plant Botanical Name	Extraction Solvent	Susceptible Microorganism	MLC/MIC
kānuka	<i>Kunzea ericoides</i>	Methanol	<i>Staphylococcus aureus</i> <i>Bacillus cereus</i>	2 4
		Ethyl acetate	<i>S. aureus</i>	2
horopito	<i>Pseudowintera colorata</i>	Methanol	<i>Candida albicans</i>	1
		Ethyl acetate		2
kāmahī	<i>Weinmannia racemosa</i>	Methanol	<i>B. cereus</i> <i>S. aureus</i>	1 8
		Ethyl acetate	<i>C. albicans</i> <i>S. aureus</i>	1 8
harakeke	<i>Phormium tenax</i>	Ethyl acetate	<i>Escherichia coli</i> <i>S. aureus</i>	1 4
patete	<i>Schefflera digitata</i>	Ethyl acetate	<i>S. aureus</i>	1
			<i>C. albicans</i>	2

* MLC—minimum lethal concentration, MIC—minimum inhibitory concentration.

The ethyl acetate extract of *K. ericoides* presented a MLC/MIC ratio of 4 against *B. cereus*. A similar result was obtained with the ethyl acetate extract of *P. tenax* against *S. aureus*. Lastly, both extracts of *W. racemosa* were only bacteriostatic against *S. aureus* as MLC/MIC = 8.

3.3. Microbial Susceptibility of Different Microorganisms

The mould *A. niger* was not susceptible to any of the extracts tested. *Escherichia coli* was also very resistant. It is commonly known that Gram-negative bacteria such as *E. coli* are less susceptible to antimicrobials than Gram-positive bacteria such as *S. aureus* and *B. cereus* [27]. It is not known precisely why the Gram-negative *E. coli* was less susceptible to the plant extracts, but it may be perhaps due to the presence of an outer membrane, which endows the bacterial surface with strong hydrophilicity and acts as a strong permeability barrier, which restricts the diffusion of hydrophobic compounds through its lipopolysaccharide covering [28,29].

4. Conclusions

The results demonstrated that 12 out of the 18 produced plant extracts exhibited antimicrobial activity against at least one of the five microorganisms tested. In term of plant species, *K. ericoides*, *P. colorata*, and *W. racemosa* were the most antimicrobial plant extracts, followed by *P. tenax*, *S. digitata*, and *P. kumeraho*, and lastly *H. stricta*, *G. ligustrifolium*, and *M. ramiflorus* did not exhibit antimicrobial properties against the five microorganisms tested. The lowest MIC obtained was 62.5 µg/mL by *K. ericoides* methanol extract against both *B. cereus* and *C. albicans*, as well as by *P. colorata* (ethyl acetate) against *S. aureus*. The effects of the extracts on microorganisms were mostly microbicidal. The mould (fungus) *Aspergillus niger* was not susceptible to any of the extracts produced and the *E. coli* was only inhibited and killed by the ethyl acetate extract from *P. tenax* with both MIC and MLC of 2000 µg/mL. The less resistant microorganisms were *S. aureus*, followed by *B. cereus* and *C. albicans*.

It is essential to conduct further studies focusing on those plants which have been used traditionally by Māori as potential sources of novel antimicrobial compounds. The results of this study can help to accommodate the emerging demand for natural, safe, and effective antimicrobial products with potential applications for human health. The possibility of using plant materials as a source of natural products with potential antimicrobial properties is a very current issue. More detailed experiments should be carried out with the active extracts, namely cytotoxicity assessment and a chemical identification (e.g., HPLC) to identify the chemical composition of the extracts.

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