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Antimicrobial Activities of Malaysian Plant Species

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Abstract

Antimicrobial activities against reference Gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria and *Candida albicans* were tested on 191 plant extracts obtained from more than 30 families of plants found in the state of Sabah, Malaysia. The plant extracts were tested by a disk-diffusion technique in which antimicrobial activity was evaluated based on the ability of the plant extracts to diffuse through agar to affect the target organisms. The extracts of *Callicarpa erioclona Schau.* (Verbenaceae), *Callicarpa farinosa Roxb.* (Verbenaceae), *Sphonodesma friflora Wright* (Verbenaceae), and *Homalium panayanum F. Villar* (Flacourticeae) exhibited antimicrobial properties worthy of further investigation.

Keywords: Antimicrobial activity, *Callicarpa erioclona*, *Callicarpa farinosa*, *Homalium panavanum*, Malaysian plants, *Siphonodesma friflora*, Verbenaceae.

Introduction

Plants have been used to treat various chronic and infectious diseases in traditional medicine and are known to contain a wide range of substances (Nimri et al., 1999). There are numerous reports on the inhibitory effects of various plant extracts on the growth of many bacteria and fungi in culture. For example, ethanol extracts of *Cassia alata* L. leaves exhibited high antimicrobial activities against various species of dermatophytic fungi (Ibrahim & Osman, 1995) and methanol extracts of *Ceanothus americanus* L. were active against selected oral pathogens (Li et al., 1997). The emergence of new and resistant strains of microbes has undermined the effectiveness of existing antimicrobial agents and hence renewed interests in the discovery of new and novel plant-derived antimicrobial compounds.

In the discovery of new bioactive compounds from plants, several approaches to the selection of plants for pharmacological screening are known. First, the random approach, which involves the collection of all plants found in the study area. Second, phytochemical targeting, which entails the collection of all members of a plant family known to be rich in bioactive compounds, and finally, the ethno-directed sampling approach, based on traditional medicinal use(s) of a plant (Cotton, 1996; Khafagi & Dewedar, 2000; Perumal Samy & Ignacimuthu, 2000; Fyhrquist et al., 2002).

In this study, we adopted the random approach in plant collection and subjected the samples (191 samples covering over 30 plant families) to antimicrobial screening using selected microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Candida albicans*, as these represent the human pathogenic microorganisms. The main aim of this study was to qualitatively identify plants that exhibited significant antimicrobial activity for further bioassay-guided isolation of active constituents.

Materials and Methods

Plant materials and preparation of extracts

Plant samples were collected from the state of Sabah, Malaysia, with the voucher specimens deposited at the Forest Research Center, Sepilok, Sandakan, Sabah. Different parts of the plants were dried separately at 40 °C. The dried materials (100–200 g) were powdered and extracted with methanol (5 × sufficient volume of methanol to cover the

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plant materials in glass columns) using a percolation method until the color faded. Methanol extracts were collected at 24, 48, 72, 120 and 168 h, filtered, evaporated at 40 °C *in vacuo* and the residues freeze-dried. The dried residues were then kept in sample bottles at −20 °C until use. Stock solutions of the crude extracts were prepared by dissolving 50 mg of the extract in 1 ml of methanol (50 mg/ml).

Test organisms and growth conditions

The microorganisms used were *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 90028, to represent human pathogenic microorganisms. Cultures were prepared from stock cultures by streaking onto nutrient agar (NA) for bacteria and Sabouraud dextrose agar (SDA) for yeast. After an overnight incubation, a single colony was used to inoculate the sterile broths, Nutrient broth (NB) and Sabouraud dextrose broth (SDB) for bacteria and yeast, respectively. The inoculated broths were then incubated overnight. The microbial cultures were diluted 10-fold in either Mueller Hinton broth (MHB) (for bacteria) or SDB (for yeast) to give a turbidity of 0.5 McFarland (about 10^6 to 10^7 CFU/ml) and further incubated for another 15 min to allow the test organisms to enter into early exponential growth phase (Jaki et al., 1999).

Qualitative antimicrobial assay

Antimicrobial activity of the plant extracts was tested using the disk-diffusion method with modifications. Suspensions of microorganism (200 µl) were added and spread evenly on 20 ml agar preset in 90-mm Petri dishes. Whatman AA antibiotic disks (6-mm diameter) were impregnated with 20 µl of plant extract stock solutions, dried under sterile conditions, and placed on MHA and SDA for bacteria and yeast, respectively. Standard disks of ampicillin (10 µg; AMP10), streptomycin (10 µg; S10), and nystatin (100 units; NS100) were used as positive controls for Gram-positive bacteria (*S. aureus* and *E. faecalis*), Gram-negative bacteria (*E. coli* and *P. aeruginosa*), and yeast (*C. albicans*), respectively, whereas methanol-impregnated disks were used as negative control. Prior to incubation at 37 °C for 24 h, the plates were kept at room temperature for 1 h to promote diffusion of the extracts into the agar (Rios et al., 1988; Fyhrquist et al., 2002). Each treatment was carried out in triplicate.

A clear zone of growth inhibition surrounding the disk indicates the presence of antimicrobial substance in the extracts. The inhibition zones were measured in triplicate and recorded as the mean diameter (mm). Strength of activities was arbitrarily classified as strong (+++) for inhibition zone diameters of more than 15.0 mm, moderate (++) for diameters between 10.0 mm and 14.9 mm, and weak (+) for diameters less than 9.5 mm.

Results and Discussion

The effects of the 191 extract toward the different strains of bacteria and yeast varied as depicted in Table 1. The extracts were mainly active against *Staphylococcus aureus* with inhibition zone diameters ranging from 8.0 to 17.0 mm as compared with ampicillin 10 µg, which gave readings from 27.0 to 35.0 mm. These plant extracts also exhibited strong antimicrobial activities against methicillin-resistant *Staphylococcus aureus* strains (MRSA) in a pilot study, with inhibition zone diameters of 15.0 mm to 22.0 mm as compared to the inhibition zone diameters of approximately 12.0 mm for vancomycin 30-µg disks (results not shown). The results indicate that the anti-MRSA activities of these plant extracts are high in relation to vancomycin and as such may prove to be useful for the treatment of infections by MRSA.

The hit rates of the plant extracts against different bacterial strains and *Candida* are summarized in Table 2. Of the extracts, 51.8% showed activity (high, moderate, and low activities) against any of the test microorganisms. This is similar to the 41.7% reported by Khafagi and Dewedar (2000) for active plants collected by the random method but lower than those for plants collected using the ethno-directed approach. Although it yields a lower hit rate compared to the ethno-directed approach, the random approach allows unexpected plants with desired activity to be identified, and this is one of the main advantages of this approach. The results also show that the plant extracts are mainly active on *Staphylococcus aureus* (Gram-positive bacteria); about 51.8% of the 191 plant extracts screened exhibited activity on *Staphylococcus aureus*. Of this 51.8%, the majority, 42.4%, was classified as weak antimicrobial activity, 7.9% as moderate, and only 1.6% (3 plants) as highly active (Table 2). On *Enterococcus faecalis* (Gram-positive bacteria), only 2.6% of the plant extracts screened exhibited activity, and the strength of antimicrobial activity of the active plant extracts is as depicted in Table 2. However, the plant extracts did not exhibit any activity on *Escherichia coli* and *Pseudomonas aeruginosa* (Gram-negative bacteria) or on *Candida albicans*. It is therefore conceivable that the plant extracts that showed antimicrobial activity against *Staphylococcus aureus* and *Enterococcus faecalis* probably interfered with the synthesis of the peptidoglycan, which is more abundant in the Gram-positive bacterial cell wall compared to the Gram-negative bacterial wall. Unlike bacteria, the cell wall of *Candida albicans* lacks peptidoglycan and instead consists of highly branched alkali-insoluble β-glucans with a higher proportion of (1–6)-β-linkages, which probably account for it not being affected by the plant extracts.

The plant extracts, which exhibited high antibacterial activity against *Staphylococcus aureus*, including methicillin-resistant strains are *Callicarpa erioclona* Schau. *Callicarpa farinosa* Roxb. and *Sphonodesma friflora* Wright from the Verbenaceae family. However, they did not show notable activity against the other three test bacteria and *Candida albicans* (Tables 1 and 3). Extracts of *Homalium panayanum*

Table 1. Antimicrobial activity of some Malaysian plants.

No.	Voucher no.	Family	Species	Part	Antimicrobial activity				
					S. aureus	*E. coli*	*P. aeruginosa*	*E. faecalis*	*C. albicans*
1	143521	Alangiaceae	*Alangium griffithii*	Bark	+	–	–	–	–
2	143521	Alangiaceae	*Alangium griffithii*	Leaf	–	–	–	–	–
3	143516	Anacardiaceae	*Buchanania insignis*	Bark	+	–	–	–	–
4	143516	Anacardiaceae	*Buchanania insignis*	Leaf	+	–	–	–	–
5	143370	Anacardiaceae	*Semicarpus leneatus*	Bark	+	–	–	–	–
6	143370	Anacardiaceae	*Semicarpus leneatus*	Leaf	+	–	–	–	–
7	143513	Annonaceae	*Artabotrys roseus*	Bark	–	–	–	–	–
8	143374	Annonaceae	*Artabotrys roseus*	Bark	–	–	–	–	–
9	143513	Annonaceae	*Artabotrys roseus*	Leaf	–	–	–	–	–
10	143374	Annonaceae	*Artabotrys roseus*	Leaf	–	–	–	–	–
11	143390	Annonaceae	*Cythostema excelsa*	Leaf	+	–	–	–	–
12	143390	Annonaceae	*Cythostema excelsa*	Stem	+	–	–	–	–
13	143377	Annonaceae	*Desmos chinensis*	Bark	–	–	–	–	–
14	143377	Annonaceae	*Desmos chinensis*	Leaf	–	–	–	–	–
15	143397	Annonaceae	*Enicosanthum erianthoides*	Bark	–	–	–	–	–
16	143397	Annonaceae	*Enicosanthum erianthoides*	Leaf	–	–	–	–	–
17	143358	Annonaceae	*Eniscosanthum grandiflorum*	Bark	+	–	–	+	–
18	143358	Annonaceae	*Eniscosanthum grandiflorum*	Leaf	–	–	–	–	–
19	133834	Annonaceae	*Goniothalamus gigantifolius*	Leaf	+	–	–	–	–
20	143362	Annonaceae	*Goniothalamus woodii*	Bark	+	–	–	–	–
21	143362	Annonaceae	*Goniothalamus woodii*	Leaf	+	–	–	+	–
22	143396	Annonaceae	*Meiogyne virgata*	Bark	+	–	–	–	–
23	143396	Annonaceae	*Meiogyne virgata*	Leaf	–	–	–	–	–
24	143506	Annonaceae	*Neouvaria acuminatiisima*	Bark	+	–	–	–	–
25	143506	Annonaceae	*Neouvaria acuminatiisima*	Leaf	–	–	–	–	–
26	143509	Annonaceae	*Orophea corymbosa*	Bark	+	–	–	–	–
27	143509	Annonaceae	*Orophea corymbosa*	Leaf	+	–	–	–	–
28	143511	Annonaceae	*Polyalthia insignis*	Bark	+	–	–	–	–
29	143511	Annonaceae	*Polyalthia insignis*	Leaf	–	–	–	–	–
30	143502	Annonaceae	*Polyalthia longipes*	Leaf	+	–	–	–	–
31	145376	Annonaceae	*Polyalthia microtus*	Bark	–	–	–	–	–
32	145376	Annonaceae	*Polyalthia microtus*	Leaf	+	–	–	–	–
33	143524	Annonaceae	*Polyalthia rumphii*	Leaf	–	–	–	–	–
34	143524	Annonaceae	*Polyalthia rumphii*	Root	–	–	–	–	–
35	143395	Annonaceae	*Popowia odoardoi*	Bark	+	–	–	–	–
36	145365	Annonaceae	*Popowia odoardoi*	Leaf	++	–	–	–	–
37	145365	Annonaceae	*Popowia odoardoi*	Stem	++	–	–	–	–
38	143368	Annonaceae	*Uvaria rufa*	Bark	+	–	–	–	–
39	143368	Annonaceae	*Uvaria rufa*	Leaf	+	–	–	–	–
40	143507	Annonaceae	*Xylopia malayana*	Bark	–	–	–	–	–

41	143507	Annonaceae	*Xylopia malayana*	Leaf	–	–	–	–	–
42	145362	Apocynaceae	*Kopsia dasyrachis*	Leaf	–	–	–	–	–
43	145362	Apocynaceae	*Kopsia dasyrachis*	Stem	–	–	–	–	–
44	143365	Araliaceae	*Arthrophyllum collinum*	Bark	–	–	–	–	–
45	143365	Araliaceae	*Arthrophyllum collinum*	Leaf	–	–	–	–	–
46	143380	Araliaceae	*Schefflera petiolosa*	Bark	–	–	–	–	–
47	143380	Araliaceae	*Schefflera petiolosa*	Leaf	–	–	–	–	–
48	145361	Burseraceae	*Canarium denticulatum*	Bark	+	–	–	–	–
49	145361	Burseraceae	*Canarium denticulatum*	Leaf	+	–	–	–	–
50	143522	Burseraceae	*Canarium hirsutum*	Bark	+	–	–	–	–
51	143522	Burseraceae	*Canarium hirsutum*	Fruit	+	–	–	–	–
52	143522	Burseraceae	*Canarium hirsutum*	Leaf	++	–	–	–	–
53	143394	Cornaceae	*Agelaea borneensis*	Bark	++	–	–	–	–
54	143394	Cornaceae	*Agelaea borneensis*	Leaf	+	–	–	–	–
55	143381	Cornaceae	*Chionanthus laxiflorus*	Bark	+	–	–	–	–
56	143381	Cornaceae	*Chionanthus laxiflorus*	Leaf	++	–	–	–	–
57	143503	Dichapetaceae	*Dichapetalum gelonoides*	Bark	–	–	–	–	–
58	143503	Dichapetaceae	*Dichapetalum gelonoides*	Leaf	+	–	–	–	–
59	143504	Ebenaceae	*Diospyros cauliflora*	Bark	–	–	–	–	–
60	143504	Ebenaceae	*Diospyros cauliflora*	Leaf	–	–	–	–	–
61	143519	Ebenaceae	*Diospyros tuberculata*	Bark	–	–	–	–	–
62	143519	Ebenaceae	*Diospyros tuberculata*	Leaf	+	–	–	–	–
63	145393	Euphorbiaceae	*Borneodendron aenigmaticum*	Bark	–	–	–	–	–
64	145393	Euphorbiaceae	*Borneodendron aenigmaticum*	Leaf	–	–	–	–	–
65	145400	Euphorbiaceae	*Mallotus griffithianus*	Bark	+	–	–	–	–
66	145400	Euphorbiaceae	*Mallotus griffithianus*	Leaf	–	–	–	–	–
67	145371	Euphorbiaceae	*Mallotus wrayi*	Leaf	+	–	–	–	–
68	145388	Flacourticeae	*Homalium panavanum*	Bark	–	–	–	++	–
69	145388	Flacourticeae	*Homalium panavanum*	Leaf	++	–	–	+++	–
70	145398	Guttiferae	*Callophyllum blancoi*	Bark	+	–	–	–	–
71	145398	Guttiferae	*Callophyllum blancoi*	Leaf	+	–	–	–	–
72	145377	Guttiferae	*Callophyllum gracilipes*	Bark	–	–	–	–	–
73	145377	Guttiferae	*Callophyllum gracilipes*	Leaf	–	–	–	–	–
74	145385	Guttiferae	*Callophyllum nodosum*	Bark	+	–	–	–	–
75	145385	Guttiferae	*Callophyllum nodosum*	Leaf	+	–	–	–	–
76	143379	Guttiferae	*Garcinia bancana*	Bark	+	–	–	–	–
77	143379	Guttiferae	*Garcinia bancana*	Leaf	+	–	–	–	–
78	133842	Guttiferae	*Garcinia brianii*	Bark	–	–	–	–	–
79	133842	Guttiferae	*Garcinia brianii*	Leaf	+	–	–	–	–
80	133846	Guttiferae	*Garcinia cuspidata*	Bark	+	–	–	–	–
81	142698	Guttiferae	*Garcinia parvifolia*	Leaf	+	–	–	–	–
82	142674	Guttiferae	*Garcinia rostrata*	Leaf	+	–	–	–	–
83	143353	Lauraceae	*Caryodaphnopsis tonkenensis*	Bark	–	–	–	–	–
84	143353	Lauraceae	*Caryodaphnopsis tonkenensis*	Leaf	–	–	–	–	–
85	145368	Lauraceae	*Litsea garciae*	Bark	–	–	–	–	–
86	145368	Lauraceae	*Litsea garciae*	Leaf	–	–	–	–	–

Table 1. Continued

No.	Voucher no.	Family	Species	Part	Antimicrobial activity				
					S. aureus	*E. coli*	*P. aeruginosa*	*E. faecalis*	*C. albicans*
87	145380	Lauraceae	*Litsea sessilis*	Leaf	–	–	–	–	–
88	143360	Leguminosae	*Spatholobus macropterus*	Bark	+	–	–	–	–
89	143360	Leguminosae	*Spatholobus macropterus*	Leaf	+	–	–	–	–
90	143356	Leguminosae	*Dalbergia parviflora*	Bark	+	–	–	–	–
91	143356	Leguminosae	*Dalbergia parviflora*	Leaf	+	–	–	–	–
92	143388	Leguminosae	*Dalbergia pseudo-sissoo*	Bark	++	–	–	–	–
93	143388	Leguminosae	*Dalbergia pseudo-sissoo*	Leaf	+	–	–	–	–
94	143392	Meliaceae	*Aglaia affinis*	Bark	++	–	–	–	–
95	143392	Meliaceae	*Aglaia affinis*	Leaf	+	–	–	–	–
96	143393	Meliaceae	*Aglaia argentea*	Bark	–	–	–	–	–
97	143393	Meliaceae	*Aglaia argentea*	Leaf	–	–	–	–	–
98	143361	Meliaceae	*Aglaia rivularis*	Bark	+	–	–	–	–
99	143361	Meliaceae	*Aglaia rivularis*	Leaf	+	–	–	–	–
100	143366	Meliaceae	*Aglaia shawiana*	Bark	+	–	–	–	–
101	143366	Meliaceae	*Aglaia shawiana*	Leaf	–	–	–	–	–
102	143512	Meliaceae	*Chisocheton erythrocarpus*	Bark	+	–	–	–	–
103	143512	Meliaceae	*Chisocheton erythrocarpus*	Leaf	–	–	–	–	–
104	145367	Meliaceae	*Chisocheton macranthus*	Bark	–	–	–	–	–
105	145367	Meliaceae	*Chisocheton macranthus*	Fruit	–	–	–	–	–
106	145367	Meliaceae	*Chisocheton macranthus*	Leaf	++	–	–	–	–
107	143513	Meliaceae	*Chisocheton pentandrus*	Bark	+	–	–	–	–
108	143513	Meliaceae	*Chisocheton pentandrus*	Leaf	–	–	–	–	–
109	143376	Meliaceae	*Chisocheton polyandra*	Bark	–	–	–	–	–
110	143376	Meliaceae	*Chisocheton polyandra*	Leaf	–	–	–	–	–
111	142663	Meliaceae	*Chisocheton polyandrus*	Leaf	–	–	–	–	–
112	143355	Meliaceae	*Dysoxylum ramiflorum*	Bark	++	–	–	–	–
113	143355	Meliaceae	*Dysoxylum ramiflorum*	Leaf	+	–	–	+	–
114	143364	Meliaceae	*Dysoxylum rugulosum*	Bark	+	–	–	–	–
115	143364	Meliaceae	*Dysoxylum rugulosum*	Leaf	+	–	–	–	–
116	145378	Menispermaceae	*Fibraurea chloroleuca*	Bark	+	–	–	–	–
117	145378	Menispermaceae	*Fibraurea chloroleuca*	Fruit	–	–	–	–	–
118	145378	Menispermaceae	*Fibraurea chloroleuca*	Leaf	–	–	–	–	–
119	143518	Moraceae	*Ficus septica*	Leaf	–	–	–	–	–
120	145397	Myrtaceae	*Decarspermum fruticosum*	Bark	++	–	–	–	–
121	145397	Myrtaceae	*Decarspermum fruticosum*	Leaf	++	–	–	–	–
122	145392	Ochnaceae	*Gomphia serrata*	Bark	+	–	–	–	–
123	145392	Ochnaceae	*Gomphia serrata*	Leaf	–	–	–	–	–
124	145396	Oleaceae	*Chionanthus crispus*	Bark	++	–	–	–	–
125	145396	Oleaceae	*Chionanthus crispus*	Leaf	+	–	–	–	–
126	143359	Piperaceae	*Piper officinarium*	Leaf	–	–	–	–	–

127	143359	Piperaceae	*Piper officinarium*	Stem	–	–	–	–	–
128	145373	Rhamnaceae	*Vantilago dichotoma*	Leaf	–	–	–	–	–
129	143525	Rhizophoraceae	*Carallia borneensis*	Leaf	–	–	–	–	–
130	145363	Rubiaceae	*Gardenia tubifera*	Bark	–	–	–	–	–
131	145363	Rubiaceae	*Gardenia tubifera*	Leaf	+	–	–	–	–
132	145395	Rubiaceae	*Morinda rigida*	Bark	–	–	–	–	–
133	145395	Rubiaceae	*Morinda rigida*	Leaf	–	–	–	–	–
134	145395	Rubiaceae	*Morinda rigida*	Stem	–	–	–	–	–
135	145364	Rubiaceae	*Praravinia suberosa*	Bark	–	–	–	–	–
136	145364	Rubiaceae	*Praravinia suberosa*	Leaf	–	–	–	–	–
137	145382	Rubiaceae	*Psychotria sarmentosa*	Leaf	–	–	–	–	–
138	145384	Rubiaceae	*Timonius flavescens*	Bark	–	–	–	–	–
139	145384	Rubiaceae	*Timonius flavescens*	Leaf	–	–	–	–	–
140	145366	Rutaceae	*Clausena excavata*	Bark	–	–	–	–	–
141	145366	Rutaceae	*Clausena excavata*	Leaf	+	–	–	–	–
142	143398	Rutaceae	*Glycosmis macrantha*	Leaf	–	–	–	–	–
143	143398	Rutaceae	*Glycosmis macrantha*	Stem	–	–	–	–	–
144	143391	Rutaceae	*Melicope accedens*	Bark	–	–	–	–	–
145	143391	Rutaceae	*Melicope accedens*	Leaf	–	–	–	–	–
146	143375	Rutaceae	*Melicope incana*	Bark	–	–	–	–	–
147	143375	Rutaceae	*Melicope incana*	Leaf	–	–	–	–	–
148	143367	Rutaceae	*Melicope luna-akenda*	Bark	–	–	–	–	–
149	143367	Rutaceae	*Melicope luna-akenda*	Leaf	+	–	–	–	–
150	145390	Rutaceae	*Melicope subunifoliata*	Bark	–	–	–	–	–
151	145390	Rutaceae	*Melicope subunifoliata*	Leaf	++	–	–	–	–
152	143389	Rutaceae	*Micromelum minutum*	Bark	+	–	–	–	–
153	143389	Rutaceae	*Micromelum minutum*	Leaf	–	–	–	–	–
154	143383	Rutaceae	*Tetractomia tetandrum*	Bark	–	–	–	–	–
155	143383	Rutaceae	*Tetractomia tetandrum*	Leaf	–	–	–	–	–
156	143357	Sapindaceae	*Harpullia arborea*	Bark	+	–	–	–	–
157	143357	Sapindaceae	*Harpullia arborea*	Leaf	–	–	–	–	–
158	143514	Sapindaceae	*Walsura pinnata*	Bark	+	–	–	–	–
159	143514	Sapindaceae	*Walsura pinnata*	Leaf	–	–	–	–	–
160	143520	Scyphostegiaceae	*Scyphostegia borneensis*	Bark	+	–	–	–	–
161	143520	Scyphostegiaceae	*Scyphostegia borneensis*	Leaf	–	–	–	–	–
162	133822	Simaroubaceae	*Quassia indica*	Bark	–	–	–	–	–
163	145381	Sterculiaceae	*Sterculia stipulata*	Bark	–	–	–	–	–
164	145381	Sterculiaceae	*Sterculia stipulata*	Leaf	–	–	–	–	–
165	145383	Theaceae	*Schima wallichi*	Bark	+	–	–	–	–
166	145383	Theaceae	*Schima wallichi*	Leaf	+	–	–	–	–
167	145391	Ulmaceae	*Gironniera subaequaelis*	Leaf	–	–	–	–	–
168	145375	Urticaceae	*Dendrocnide elliptica*	Bark	–	–	–	–	–
169	145375	Urticaceae	*Dendrocnide elliptica*	Leaf	–	–	–	–	–
170	143354	Urticaceae	*Dendrocnide stimulans*	Bark	++	–	–	–	–
171	143354	Urticaceae	*Dendrocnide stimulans*	Leaf	–	–	–	–	–
172	145387	Urticaceae	*Leucosyke winklerii*	Bark	+	–	–	–	–

Table 1. Continued

No.	Voucher no.	Family	Species	Part	Antimicrobial activity				
					S. aureus	*E. coli*	*P. aeruginosa*	*E. faecalis*	*C. albicans*
173	145387	Urticaceae	*Leucosyke winklerii*	Leaf	+	–	–	–	–
174	143363	Urticaceae	*Oreocnide frinervis*	Bark	+	–	–	–	–
175	143363	Urticaceae	*Oreocnide frinervis*	Leaf	+	–	–	–	–
176	106702	Verbenaceae	*Callicarpa erioclona*	Bark	+++	–	–	–	–
177	133850	Verbenaceae	*Callicarpa fulfuraceae*	Bark	+++	–	–	–	–
178	133849	Verbenaceae	*Callicarpa fulvohirsuta*	Bark	+	–	–	–	–
179	143378	Verbenaceae	*Callicarpa havilandii*	Bark	+	–	–	–	–
180	143378	Verbenaceae	*Callicarpa havilandii*	Leaf	+	–	–	–	–
181	145389	Verbenaceae	*Callicarpa havilandii*	Bark	+	–	–	–	–
182	145389	Verbenaceae	*Callicarpa havilandii*	Leaf	+	–	–	–	–
183	145374	Verbenaceae	*Callicarpa longifolia*	Leaf	–	–	–	–	–
184	106701	Verbenaceae	*Callicarpa stapfii*	Bark	+	–	–	–	–
185	106701	Verbenaceae	*Callicarpa stapfii*	Leaf	+	–	–	–	–
186	143372	Verbenaceae	*Sphonodesma friflora*	Bark	+	–	–	–	–
187	143372	Verbenaceae	*Sphonodesma friflora*	Leaf	+++	–	–	–	–
188	145386	Verbenaceae	*Stachytarpeta jamaicencis*	Stem	+	–	–	–	–
189	145370	Vitaceae	*Leea indica*	Bark	–	–	–	–	–
190	145370	Vitaceae	*Leea indica*	Leaf	+	–	–	–	–
191	145379	Zingerberaceae	*Alpinia fraseriana*	Stem	–	–	–	–	–
			Methanol (negative control)		–	–	–	–	–
			Ampicillin (positive control)		+++		+++		
			Streptomycin (positive control)			+++		+++	
			Nystatin (positive control)						+++

–: no inhibition; +: inhibition diameter of less than 9.5 mm; ++: inhibition diameter between 10.0 to 14.9 mm; +++: inhibition diameter of more than 15.0 mm; disk diameter: 6 mm. Each extract (1 mg/disk) was tested in triplicate.

Table 2. Percentage of active extracts.

	S. aureus	*E. coli*	*P. aeruginosa*	*E. faecalis*	*C. albicans*	Active on one or more bacteria and yeast
Total samples tested	191	191	191	191	191	–
Percentage active, %	51.8 [99]	0	0	2.6 [5]	0	51.8
Highly active (+++), %	1.6 [3]	0	0	0.5 [1]	0	–
Moderately active (++), %	7.9 [15]	0	0	0.5 [1]	0	–
Weakly active (+), %	42.4 [81]	0	0	1.6 [3]	0	–

[ ]: number of plant extracts.

Table 3. Summary of plants exhibiting high and moderate antimicrobial activities against *S. aureus*.

No.	Voucher no.	Family	Species	Part	Antimicrobial activity *S. aureus*
1	106702	Verbenaceae	*Callicarpa erioclona*	Bark	+++
2	133850	Verbenaceae	*Callicarpa fulfuraceae*	Bark	+++
3	143372	Verbenaceae	*Sphonodesma friflora*	Leaf	+++
4	145365	Annonaceae	*Popowia odoardoi*	Leaf	++
5	145365	Annonaceae	*Popowia odoardoi*	Stem	++
6	143522	Burseraceae	*Canarium hirsutum*	Leaf	++
7	143394	Cornaceae	*Agelaea borneensis*	Bark	++
8	143381	Cornaceae	*Chionanthus laxiflorus*	Leaf	++
9	145388	Flacourticeae	*Homalium panavanum*	Leaf	++
10	143388	Leguminosae	*Dalbergia pseudo-sissoo*	Bark	++
11	143392	Meliaceae	*Aglaia affinis*	Bark	++
12	145367	Meliaceae	*Chisocheton macranthus*	Leaf	++
13	143355	Meliaceae	*Dysoxylum ramiflorum*	Bark	++
14	145397	Myrtaceae	*Decarspermum fruticosum*	Bark	++
15	145397	Myrtaceae	*Decarspermum fruticosum*	Leaf	++
16	145396	Oleaceae	*Chionanthus crispus*	Bark	++
17	145390	Rutaceae	*Melicope subunifoliata*	Leaf	++
18	143354	Urticaceae	*Dendrocnide stimulans*	Bark	++

++: inhibition diameter between 10.0 to 14.9 mm; +++: inhibition diameter of more than 15.0 mm; disk diameter: 6 mm. Each extract (1 mg/disk) was tested in triplicate.

Table 4. A summary of plants exhibiting high, moderate, and weak antimicrobial activities against *E. faecalis*.

No.	Voucher no.	Family	Species	Part	Antimicrobial activity *E. faecalis*
1	145388	Flacourticeae	*Homalium panavanum*	Leaf	+++
2	145388	Flacourticeae	*Homalium panavanum*	Bark	++
3	143358	Annonaceae	*Eniscosanthum grandiflorum*	Bark	+
4	143362	Annonaceae	*Goniothalamus woodii*	Leaf	+
5	143355	Meliaceae	*Dysoxylum ramiflorum*	Leaf	+

+: inhibition diameter of less than 9.5 mm; ++: inhibition diameter between 10.0 to 14.9 mm; +++: inhibition diameter of more than 15.0 mm; disk diameter: 6 mm. Each extract (1 mg/disk) was tested in triplicate.

F. Villar (Flacourticeae) from leaves and bark exhibited high bactericidal activities on *Enterococcus faecalis* and moderate activities on *Staphylococcus aureus* (Tables 1, 3, and 4). These active plants have now been selected for further testing and bioassay-guided fractionation to ascertain the extent of their antimicrobial activities and to identify the active constituents. The effect of the active constituents would be extended to cover clinical strains of *Staphylococcus aureus*, particularly, methicillin-resistant strains.

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