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Volatile Constituents and Antimicrobial Activity of Naio (*Myoporum sandwicense* A. Gray), a Native Hawaiian Tree

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Abstract: *Myoporum sandwicense* A. Gray (naio) is one of the characteristic trees of Hawaiian montane–subalpine mesic forests. In this study, lab-distilled oils of *M. sandwicense* leaves, wood, and twigs growing on the island of Hawaii, as well as industrially produced wood oils, were characterized by gas chromatography–mass spectrometry (GC-MS). The lab-distilled oils were screened for antimicrobial activity. *M. sandwicense* leaf essential oil was rich in β-caryophyllene (15.1%), α-humulene (12.8%), germacrene D (7.9%), bicyclogermacrene (12.5%), brigalow ketol (9.6%), and myoporone (16.8%), while the wood essential oils were dominated by α-bisabolol and *trans*-α-bisabolol oxide B. The sapwood oil was dominated by palmitic acid (35.5%), linoleic acid (19.7%), oleic acid (31.9%), and stearic acid (5.7%), whereas the oil from twigs was rich in tricosane (77.3%) and pentacosane (13.1%). *M. sandwicense* essential oils were screened for antimicrobial activity against a panel of potentially pathogenic bacteria and fungi. The leaf essential oil of *M. sandwicense* showed excellent antibacterial activity against *S. pyogenes* and antifungal activity against *A. fumigatus*. The wood essential oil showed notable activity against *S. pyogenes*, *A. fumigatus*, *A. niger*, and *M. gypseum*. The twig oil was remarkably active against mold species. This work is the first report we are aware of on the composition and antimicrobial properties of naio essential oils.

Keywords: essential oil; chemical composition; naio; *Myoporum sandwicense*; α-bisabolol; myoporone; furanosesquiterpenoid

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1. Introduction

Myoporum sandwicense A. Gray (Scrophulariaceae), commonly known as "Naio", "false sandalwood", or "bastard sandalwood", is a notoriously polymorphic evergreen shrub or tree that can grow as a creeping, prostrate shrub or it may occur as a tree around 15 m tall [1]. Furthermore, there are several varieties of *M. sandwicense* (e.g., *M. sandwicense* var. *sandwicense*, *M. sandwicense* var. *fauriei*, *M. sandwicense* var. *st.-johnii*, and *M. sandwicense* var. *wilder* [1]. *Myoporum sandwicense* is one of the characteristic trees of Hawaiian montane–subalpine mesic forests where it occurs with *Metrosideros polymorpha* Gaudich. (ōhi'a), *Acacia koa* A. Gray (koa), *Sapindus saponaria* L. (a'e), *Nestegis sandwicensis* (A. Gray) O. Deg., I. Deg. & L.A.S. Johnson (olopua), and *Sophora chrysophylla* Seem. (māmane) [2]. On the island of Hawai'i, both *M. sandwicense* var. *fauriei*, and *M. sandwicense* var. *st.-johnii*, are found on the leeward slopes of Mauna Kea and Mauna Loa [1]. The fruits of *M. sandwicense* are important food sources for the Hawaiian thrush (omao, *Phaeornis obscurus obscurus*) [3] and were apparently the exclusive food source of the now-extinct Kona grosbeak (*Chloridops kona*) [4]. *Myoporum sandwicense* populations have been adversely affected by habitat damage by feral ungulates [5] and by an introduced insect. *Klambothrips myopori* Mound and Morris (Phlaeothripidae) is a potential threat to the tree form of naio [6].

We have been interested in examining the essential oils of tropical plants for potential cultivation for the fragrance industry [7,8]. The purpose of this work was to obtain the

essential oils from the leaves, twigs, and wood of *M. sandwicense* growing on the island of Hawai'i, to chemically characterize the essential oils by gas chromatography–mass spectrometry, and to screen them for antimicrobial activity. This work is the first report we are aware of on the composition and antimicrobial properties of Naio essential oils.

2. Materials and Methods

2.1. Essential Oil Extraction

The plant materials were collected in September of 2019 from cultivated trees in South Kona, east of Kealakekua, Hawai'i (19°30′48.31″ N, 155°48′49.53″ W, elev. 1421 m). Plant identification was verified by comparison with samples from the New York Botanical Garden Virtual Herbarium (https://sweetgum.nybg.org/science/vh/specimen-list/?SummaryData=Myoporum%20sandwicense; accessed on 9 January 2023) Each plant part (leaves, twigs, wood) was shredded and steam distilled for 4–7 h using a Clevenger-type apparatus. Twenty-three *M. sandwicense* volatile oils (N1-N23) produced in industrial settings were obtained from the collection of the Aromatic Plant Research Center (APRC, Lehi, UT, USA) in 2021. Wood industrial distillation settings included a steam flow of 100 L/h, pressure of 1–3 psi, steam flow increase rate of 0.15 L/h, hydrolyte temp of 55 °C, and duration of 3–5 days.

2.2. Gas Chromatography–Mass Spectrometry

M. sandwicense essential oils were analyzed by gas chromatography–mass spectrometry (GC-MS) using a Shimadzu GCMS-QP2010 Ultra operated in the electron impact (EI) mode (electron energy = 70 eV), scan range = 40–400 atomic mass units, scan rate = 3.0 scans/s, and GC-MS solution software v. 4.20 (Shimadzu Scientific Instruments, Columbia, MD, USA). The GC column was a ZB-5 ms fused silica capillary column (Phenomenex, Torrance, CA, USA) with a (5% phenyl)-polymethylsiloxane stationary phase and a film thickness of 0.25 µm. The carrier gas was helium, with a column head pressure of 552 kPa and a flow rate of 1.37 mL/min. The injector temperature was 260 °C and the ion source temperature was 260 °C. The GC oven temperature was programmed for 50 °C initial temperature; temperature increased at a rate of 2 °C/min to 260 °C. A 5% *w*/*v* solution of the sample in CH_2Cl_2 was prepared, and 0.1 µL was injected with a splitting mode (30:1). Identification of the oil components was based on their retention indices, determined by reference to a homologous series of *n*-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the databases [9–12].

2.3. Hierarchical Cluster Analysis

M. sandwicense wood oils obtained from trusted industrial suppliers were used in the cluster analysis. The essential oil compositions were treated as operational taxonomic units (OTUs). The percentages of the major components (α-bisabolol, α-bisabolol oxide B, unidentified (1699), unidentified (1666), β-bisabolene, fokienol, dendrolasin, 6-*epi*-α-bisabolol oxide B, (*E*)-α-bisabolene, (*E*)-β-farnesene, himachal-2-en-7β-ol, (*E*)-nerolidol, limona ketone, and β-sesquiphellandrene) were used to determine the chemical associations between the essential oils using agglomerative hierarchical cluster (AHC) analysis using XLSTAT Premium, version 2018.5.53172 (Addinsoft, Paris, France). Dissimilarity was determined using Euclidean distance, and clustering was defined using Ward's method.

2.4. Antimicrobial Screening

M. sandwicense essential oils were screened for antimicrobial activity against Gram-positive bacteria (*Bacillus cereus* (ATCC No. 14579), *Cutibacterium acnes* (ATCC No. 11827), *Staphylococcus aureus* (ATCC No. 29213), *Staphylococcus epidermidis* (ATCC No. 12228), *Streptococcus pyogenes* (ATCC No. 19615), and *Streptococcus pneumoniae* (ATCC No. 49136)), Gram-negative bacteria (*Escherichia coli* (ATCC No. 25922), *Pseudomonas aeruginosa* (ATCC No. 27853), *Serratia marcescens* (ATCC No. 14756), *Helicobacter pylori* (ATCC No. 51111), and *Salmonella enterica subsp. enterica* serovar *Typhimurium* (ATCC No. 14028)), molds

(*Aspergillus niger* (ATCC No. 16888), *Aspergillus fumigatus* (ATCC No. 96918), *Microsporum canis* (ATCC No. 11621), *Microsporum gypseum* (ATCC No. 24102), and *Trichophyton mentagrophytes* (ATCC No. 18748)), and yeasts (*Cryptococcus neoformans* (ATCC No. 32045) and *Candida albicans* (ATCC No. 18804)) using the microbroth dilution technique, as previously reported [13].

All bacteria were cultured on tryptic soy agar (Sigma-Aldrich, St. Louis, MO, USA) except for *H. pylori* and *Streptococcus pneumoniae*, which were grown on tryptic soy agar supplemented with 7% (*v*/*v*) defibrillated whole sheep blood (Cleveland Scientific, Ohio, USA) under micro-aerophilic conditions for 3 days [14]. All fungi were cultured on yeast malt agar (Sigma-Aldrich, St. Louis, MO, USA). For bacteria, a 50-µL volume of 1% (*w*/*v*) solution of the samples in DMSO was diluted in 50 µL of cation-adjusted Mueller–Hinton broth (CAMHB) (Sigma-Aldrich, St. Louis, MO, USA). The sample solutions were then serially diluted (1:1) in fresh CAMHB to obtain concentrations of 2500, 1250, 625, 313, 156, 78, 39, and 20 µg/mL. The microbes were harvested from a fresh culture and added to each well at a concentration of approximately 1.5×10^8 colony-forming unit (CFU)/mL for bacteria, and 7.5×10^7 CFU/mL for fungi. The 96-well microdilution plates for bacteria were incubated at 37 °C, and the fungi were incubated at 35 °C for 24 h. The minimum inhibitory concentration (MIC) was determined as the lowest concentration with no turbidity. Gentamicin (Sigma-Aldrich, St. Louis, MO, USA) was used as a positive antibiotic control, and DMSO was used as the negative control (50 µL DMSO diluted in 50 µL broth medium, and then serially diluted, as above). For fungi, the above-mentioned method was implemented using yeast nitrogen base growth medium (Sigma-Aldrich, St. Louis, MO, USA) and Amphotericin B (Sigma-Aldrich, St. Louis, MO, USA) as a positive antifungal control.

3. Results and Discussion

3.1. Essential Oil Composition

Steam distillation of the leaves of *M. sandwicense* produced a yield of 0.11%. Analysis of the leaf essential oil by GC-MS showed the oil to be rich in the sesquiterpene hydrocarbons β-caryophyllene (15.1%), α-humulene (12.8%), germacrene D (7.9%), bicyclogermacrene (12.5%), and the furanoterpenoids brigalow ketol (9.6%) and myoporone (16.8%) (Table 1).

Table 1. Essential oil composition of *Myoporum sandwicense* leaf essential oil.

RI [a]	Compound	%	RI [a]	Compound	%
1025	Limonene	0.1	1579	Globulol	0.6
1095	Linalool	tr [b]	1588	Viridiflorol	0.4
1326	δ-Elemene	0.9	1590	Cubeban-11-ol	0.1
1370	α-Copaene	0.3	1600	Rosifoliol	0.1
1376	*cis*-β-Elemene	0.2	1603	Humulene epoxide II	0.4
1382	β-Cubebene	0.2	1617	5-*epi*-7-*epi*-β-Eudesmol	0.1
1384	*trans*-β-Elemene	3.9	1621	1-*epi*-Cubenol	0.1
1414	β-Caryophyllene	15.1	1625	*iso*-Spathulenol	0.4
1424	β-Copaene	0.1	1635	τ-Cadinol	0.2
1433	Aromadendrene	0.2	1637	τ-Muurolol	0.2
1446	(*E*)-β-Farnesene	0.2	1639	Unidentified [c]	0.7
1450	α-Humulene	12.8	1648	α-Cadinol	1.0
1469	*cis*-Murrola-4 (14),5-diene	0.2	1652	Unidentified [d]	1.2
1475	Germacrene D	7.9	1703	9-Oxodendrolasin	0.2
1483	β-Selinene	0.3	1707	Unidentified [e]	0.5
1485	Viridiflorene (=Ledene)	0.4	1716	Brigalow ketol	9.6
1490	Bicyclogermacrene	12.5	1739	1-[5-(3-Furyl)-2-methyltetrahydro-2-furanyl]-4-methyl-3-penten-2-one	0.8

Table 1. *Cont.*

RI [a]	Compound	%	RI [a]	Compound	%
1492	α-Muurolene	0.2	1745	Unidentified [f]	1.3
1501	Germacrene A	0.1	1754	(*Z*)-Dihydrophymaspermone	0.2
1506	γ-Cadinene	0.2	1817	(*E*)-Dihydrophymaspermone	0.1
1511	δ-Cadinene	1.0	1826	Perillup ketol	1.6
1526	*trans*-Cadina-1,4-diene	0.0	1834	Unidentified [g]	0.6
1530	α-Cadinene	0.1	1882	(*E*)-Hexadecantrienal	0.1
1541	Elemol	0.3	1893	Myoporone	16.8
1553	(*E*)-Nerolidol	1.0		Monoterpene hydrocarbons	0.1
1562	Dendrolasin	1.1		Oxygenated monoterpenoids	tr [b]
1563	Palustrol	0.1		Sesquiterpene hydrocarbons	56.8
1570	Spathulenol	0.6		Oxygenated sesquiterpenoids	36.6
1575	Caryophylene oxide	0.4		Total identified	93.5

[a] Retention index determined with respect to a homologous series of *n*-alkanes on a ZB-5 ms column. [b] Trace (<0.05%). [c] MS: 250 (5%), 232 (11%), 199 (21%), 171 (26%), 161 (38%), 147 (41%), 131 (42%), 125 (39%), 110 (39%), 95 (100%), 83 (54%), 71 (34%), 59 (62%), 57 (56%), 43 (97%), 41 (57%). [d] MS: 232 (2%), 214 (85%), 199 (58%), 185 (28%), 171 (34%), 157 (40%), 143 (73%), 129 (60%), 122 (68%), 107 (100%), 95 (100%), 81 (44%), 77 (49%), 67 (30%), 55 (36%), 43 (46%), 41 (48%). [e] MS: 230 (74%), 215 (29%), 201 (35%), 187 (25%), 159 (40%), 145 (29%), 131 (23%), 105 (21%), 96 (35%), 83 (60%), 82 (54%), 69 (38%), 68 (39%), 67 (36%), 57 (72%), 55 (100%), 43 (85%), 41 (71%). [f] MS: 232 (3%), 122 (46%), 107 (100%), 95 (18%), 91 (8%), 79 (8%), 67 (13%), 55 (7%), 43 (7%), 41 (11%). [g] MS: 230 (2%), 215 (5%), 193 (16%), 147 (45%), 95 (100%), 83 (78%), 55 (38%), 41 (9%).

Two samples of *M. sandwicense* wood were steam-distilled in a lab setting and analyzed by GC-MS (Table 2). The average oil yield was 0.34%. The oil was yellow to golden in color and had a woody, sweet, slightly spicy, and sandalwood-like aroma. Twenty-three industrially produced *M. sandwicense* wood oils (N1–N23) were analyzed by GC-MS (Table 3). Both lab-distilled and industrially distilled *M. sandwicense* wood essential oils were dominated by α-bisabolol and *trans*-α-bisabolol oxide B. Based on *M. sandwicense* essential oil compositions, a hierarchical cluster analysis of the oils from this work was carried out. The dissimilarity index was very small, indicating no significant differences in the essential oil compositions of the tested samples (Figure 1).

Table 2. Essential oil compositions of lab-distilled *Myoporum sandwicense* wood essential oils.

RI_{calc} [a]	Compound	Sample 1 (%)	Sample 2 (%)
1131	Limona ketone	tr [b]	0.12
1452	(*E*)-β-Farnesene	0.14	0.12
1467	Dehydrosesquicineole	tr	0.12
1488	β-Selinene	tr	—
1500	(*Z*)-α-Bisabolene	—	tr
1507	β-Bisabolene	0.83	1.33
1523	β-Sesquiphellandrene	tr	tr
1540	(*E*)-α-Bisabolene	0.14	0.36
1560	(*E*)-Nerolidol	tr	tr
1568	Dendrolasin	0.42	0.72
1593	Fokienol	0.14	—
1647	Himachal-2-en-7β-ol	—	0.12
1655	*cis*-α-Bisabolol oxide B	1.25	0.97
1659	*trans*-α-Bisabolol oxide B	23.02	19.66
1663	Unidentified [c]	2.08	4.22
1668	Intermedeol	0.42	—
1686	*epi*-α-Bisabolol	1.25	0.36
1690	α-Bisabolol	64.08	71.05
1692	Unidentified [d]	5.69	—

Table 2. *Cont.*

RI_{calc} [a]	Compound	Sample 1 (%)	Sample 2 (%)
1716	(2*E*,6*E*)-Farnesol	0.42	0.72
1764	α-Bisabolol oxide A	0.14	0.12
	Total identified	92.23	95.8

[a] Retention index determined with respect to a homologous series of *n*-alkanes on a ZB-5 ms column. [b] Trace (<0.05%). [c] MS: 230 (30%), 148 (100%), 131 (47%), 121 (15%), 105 (21%), 95 (33%), 93 (32%), 91 (46%), 81 (39%), 53 (23%), 41 (20%). [d] MS: 139 (35%), 138 (7%), 121 (8%), 95 (44%), 82 (48%), 71 (25%), 67 (15%), 55 (8%), 43 (100%).

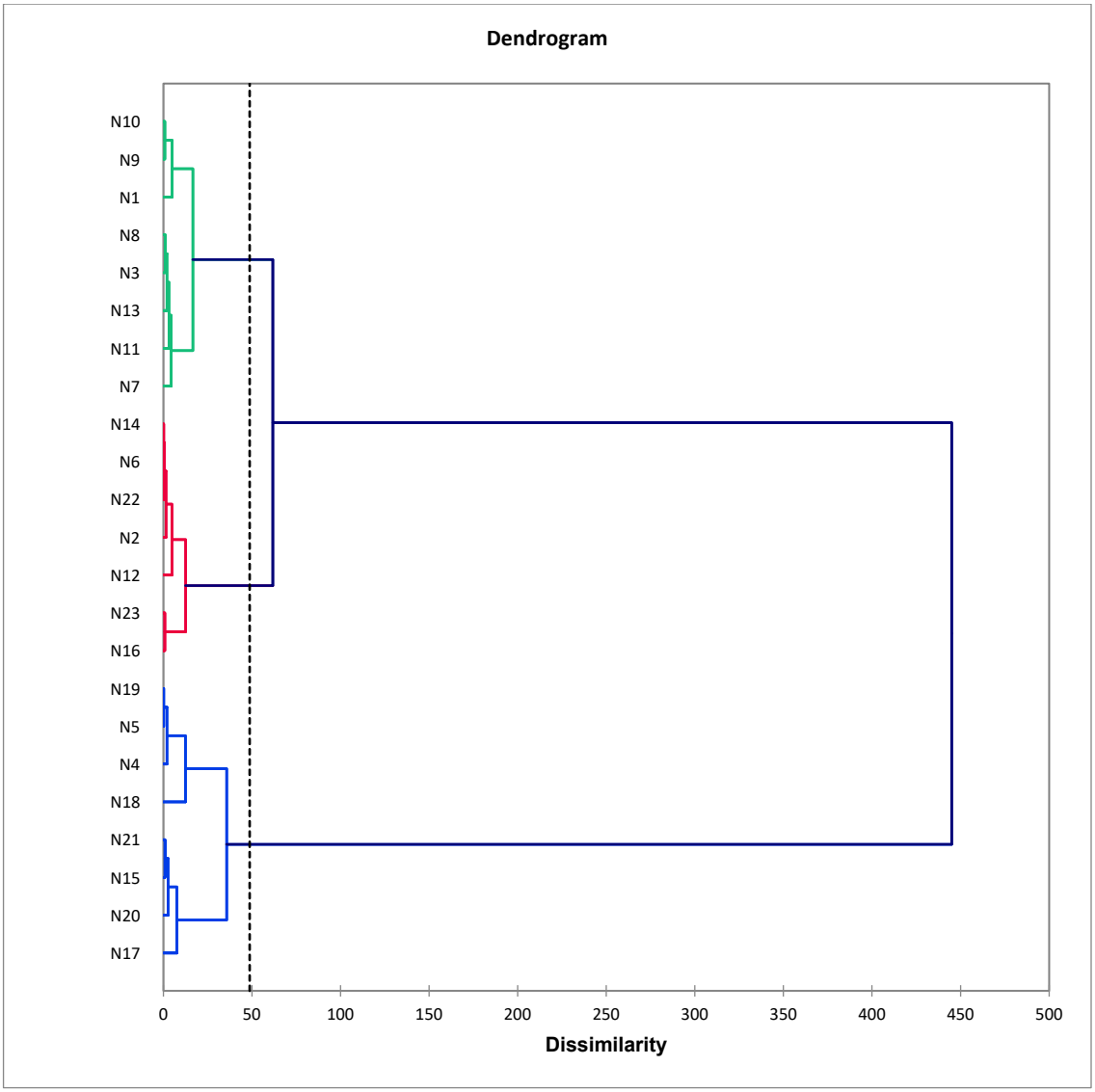

Figure 1. Agglomerative hierarchical cluster (AHC) analysis of *Myoporum sandwicense* essential oil compositions.

Steam distillation of a sample of sapwood and a sample of twigs did not produce a separable essential oil. The aqueous distillate (hydrosol) was extracted with dichloromethane, however, to provide vanishingly small quantities of volatiles that could be analyzed by GC-MS (Table 4). The sapwood essential oil was dominated by fatty acids, palmitic acid (35.5%), linoleic acid (19.7%), oleic acid (31.9%, and stearic acid (5.7%), and derivatives of fatty acids. The essential oil from the twigs was rich in *n*-alkanes, tricosane (77.3%), and pentacosane (13.1%), probably reflecting a waxy coating on the twigs.

Table 3. Essential oil compositions (%) of industrially-distilled *Myoporum sandwicense* wood essential oils.

RI_{cal}	RI_{db}	Compound	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	N13	N14	N15	N16	N17	N18	N19	N20	N21	N22	N23
1030	1030	Limonene	—	—	—	—	tr	—	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	—	tr	—	—	tr	tr	—
1098	1107	2-Methylbenzofuran	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
1132	1131	Limona ketone	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
1355	1352	Tricycloekasantalal	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	0.1	tr	tr	tr	tr
1432	1432	*trans*-α-Bergamotene	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
1448	1447	Geranyl acetone	—	—	—	—	tr	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1452	1452	(*E*)-β-Farnesene	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.2
1465	1472	Eudesma-1,4 (15),11-triene	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
1466	1466	Dehydrosesquicineole	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
1478	1478	γ-Curcumene	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
1481	1480	α-Curcumene	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
1495	1496	α-Zingiberene	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
1501	1503	(*Z*)-α-Bisabolene	tr	tr	tr	tr	0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	0.1	0.1
1504	1505	(*E*,*E*)-α-Farnesene	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	—	tr	tr	tr	tr	tr	—	tr	tr	tr	tr	tr
1508	1508	β-Bisabolene	1.1	1.1	1.2	1.2	1.3	1.2	1.2	1.2	1.2	1.1	1.1	1.3	1.3	1.3	1.2	1.1	1.4	1.4	1.1	1.0	1.3	1.4	1.3
1511	1511	(*Z*)-γ-Bisabolene	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
1514	1517	Sesquicineole	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
1524	1523	β-Sesquiphellandrene	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
1527	1528	(*E*)-γ-Bisabolene	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
1541	1541	(*E*)-α-Bisabolene	0.4	0.4	0.4	0.4	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.5	0.5	0.4	0.4	0.4	0.5	0.4	0.4	0.3	0.5	0.5	0.5
1561	1561	(*E*)-Nerolidol	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
1569	1568	Dendrolasin	0.4	0.4	0.4	0.5	0.5	0.5	0.5	0.5	0.4	0.4	0.4	0.4	0.5	0.4	0.5	0.4	0.6	0.6	0.5	0.4	0.5	0.5	0.4
1595	1596	Fokienol	0.9	1.0	1.1	0.8	0.8	0.9	1.2	1.0	1.1	1.0	1.1	1.1	1.0	0.8	0.7	0.9	0.9	0.6	0.7	0.7	1.0	1.0	1.0
1609	1611	β-Atlantol	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	tr	tr	tr	—	—	—	—	—	—	—
1640	1638	Gossonorol	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	tr	—	—	—	—
1647	1646	Himachal-2-en-7β-ol	0.2	0.2	0.2	0.1	0.2	0.1	0.2	0.2	0.2	0.1	0.2	0.2	0.3	0.2	0.1	0.1	0.2	0.2	0.2	0.1	0.2	0.2	0.1
1657	—	6-*epi*-α-Bisabolol oxide B	0.4	0.4	0.4	0.3	0.4	0.4	0.3	0.3	0.3	0.3	0.5	0.5	0.5	0.5	0.5	0.4	0.5	0.8	0.6	0.4	0.4	0.4	0.4
1661	1656	α-Bisabolol oxide B	17.7	21.9	19.2	29.6	28.1	23.0	18.7	19.0	18.1	17.6	20.4	23.3	18.4	22.6	27.5	21.2	25.6	31.0	28.1	26.4	26.3	22.5	20.8
1666	—	Unidentified	4.4	3.8	4.5	1.6	1.2	3.4	5.2	3.8	5.5	5.5	3.3	3.0	3.6	3.2	1.8	3.5	2.7	1.3	1.2	1.0	2.1	2.9	3.3
1668	1666	(*E*)-Bisabol-11-ol	0.1	tr	0.1	—	0.2	tr	—	—	tr	0.1	0.1	0.1	0.1	0.1	tr	tr	—	—	0.1	tr	—	tr	0.2
1672	1669	*epi*-α-Bisabolol	0.1	tr	0.1	tr	0.2	0.1	0.1	tr	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	—	—	0.1	0.1	tr	tr	0.1
1691	1688	α-Bisabolol	68.1	63.3	64.7	58.6	59.1	63.9	63.2	66.0	65.3	66.3	63.9	61.7	64.6	64.3	61.4	66.1	59.5	55.9	59.7	63.0	61.6	63.5	65.0
1699	—	Unidentified	3.9	4.9	5.0	4.9	4.9	4.1	6.4	5.3	5.2	4.5	5.6	5.4	6.4	4.0	3.7	3.5	5.3	5.3	4.8	4.5	4.0	4.4	3.8
1715	1716	(2*Z*,6*Z*)-Farnesol	0.2	0.2	0.2	0.3	0.1	tr	tr	—	tr	—	0.4	0.2	0.2	0.2	0.3	0.3	0.1	0.1	0.3	0.3	tr	0.1	0.1
1721	1720	(2*Z*,6*E*)-Farnesol	0.1	tr	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	tr	0.1	0.1	0.1	0.1	0.1	0.3	0.2	0.1	tr	0.1	0.1	0.2
1736	1743	(6*E*)-9-(3-Furyl)-2,6-dimethyl-2,6-nonadien-4-one	tr	tr	tr	tr	—	tr	tr	tr	tr	tr	0.1	tr	0.1	tr	0.1	0.1	tr	tr	—	—	tr	—	—
1762	1748	α-Bisabolol oxide A	—	—	—	—	—	—	—	—	—	—	tr	tr	tr	tr	tr	tr	—	—	—	—	—	—	—
1820	1824	Avocadynofuran	tr	tr	—	—	—	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
1959	1958	Palmitic acid	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	tr	tr	—	—	—	—	—
2100	2100	Heneicosane	0.1	0.1	0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	0.1
		Total identified	90.0	89.4	88.6	92.2	92.2	91.1	86.3	89.2	87.6	88.0	89.1	89.8	88.1	91.4	93.3	91.5	90.1	91.7	92.2	93.1	92.2	90.8	90.7

RI_{calc} = Retention index calculated with respect to a homologous series of n-alkanes on a ZB-5 ms column. RI_{db} = Retention index value from the databases. tr = trace (<0.05%).

Table 4. Volatile components from the sapwood and twigs of *Myoporum sandwicense*.

RI [a]	Compound	Sapwood	Twigs
746	3-Hydroxy-2-butanone	—	0.1
801	Hexanal	0.1	—
828	Furfural	—	0.1
971	Hexanoic acid	—	0.2
1028	Limonene	—	tr [b]
1099	Linalool	—	0.1
1105	Nonanal	0.1	—
1167	Octanoic acid	0.1	—
1194	α-Terpineol	—	0.1
1262	Nonanoic acid	—	tr
1318	(*E*,*E*)-Decadienal	tr	—
1358	γ-Nonalactone	—	tr
1476	9-Oxononanoic acid	0.5	—
1558	Dodecanoic acid	0.2	tr
1600	Hexadecane	—	tr
1659	Selin-11-en-4α-ol	0.1	—
1667	(*Z*)-1,7-Heptadecadiene	—	0.1
1671	(*Z*,*Z*,*Z*)-1,8,11,14-Heptadecatetraene	—	0.1
1687	α-Bisabolol	0.1	—
1691	1-Heptadecene	—	tr
1700	Heptadecane	—	tr
1714	Pentadecanal	0.1	0.1
1757	Myristic acid	0.6	tr
1800	Octadecane	—	0.1
1815	Hexadecanal	—	tr
1839	Phytone	—	0.1
1842	Cyclopentadecanolide	0.8	—
1858	Pentadecanoic acid	0.4	—
1885	(*Z*)-Hexadecatrienal	0.1	—
1892	(9*Z*)-Hexadecenal	0.1	0.1
1900	Nonadecane	—	0.1
1938	Palmitoleic acid	0.7	—
1953	(3*Z*)-Cembrene A	0.1	—
1964	Palmitic acid	35.7	2
1991	Ethyl Palmitate	—	0.2
2000	Eicosane	—	0.2
2019	(*E*,*E*)-Geranyl linalool	—	0.1
2045	Heptadecenoic acid	0.7	—
2059	Heptadecanoic acid	0.5	—
2092	1-Heneicosene	—	0.1
2100	Heneicosane	0.3	1.6
2128	(*Z*,*Z*)-Linoleic acid	19.7	0.5
2131	(*Z*,*Z*,*Z*)-Linolenic acid	—	0.2
2138	(*Z*)-Oleic acid	31.9	0.2
2156	Ethyl stearate	—	0.1
2162	Ethyl linolenate	—	0.1
2164	Stearic acid	5.7	—
2192	1-Docosene	—	0.2
2200	Docosane	0.1	1.2
2223	Nonadecadienoic acid	0.2	—
2261	Nonadecanoic acid	0.1	—
2277	(*Z*,*Z*,*Z*)-8,11,14-Eicosatrienoic acid	0.1	—
2293	1-Tricosene	—	0.1
2294	(4*E*,8*E*,12*E*)-4,9,13,17-Tetramethyloctadeca-4,8,12,16-tetraenal	0.1	—
2300	Tricosane	0.2	77
2361	Eicosanoic acid	0.2	—

Table 4. *Cont.*

RI [a]	Compound	Sapwood	Twigs
2392	1-Tetracosene	—	0.1
2400	Tetracosane	0.1	1.7
2428	Docosanal	0.1	—
2493	1-Pentacosene	—	tr
2500	Pentacosane	0.1	13.1
2600	Hexacosane	0.1	0.1
2631	Tetracosanal	—	tr
2700	Heptacosane	tr	—
	Total Identified	100.0	100.0

[a] Retention index determined with respect to a homologous series of *n*-alkanes on a ZB-5 ms column. [b] Trace (<0.05%).

There have been several previous investigations on essential oils of *Myoporum* species reported in the literature. Furanosesquiterpenoids have generally been the dominant constituents (see Table 5).

Table 5. Major components of *Myoporum* essential oils reported in the literature.

Myoporum Species	Essential Oil	Major Components	Reference
M. crassifolium Forst. f.	Wood	*epi*-α-Bisabolol (65.1%), bisabolol oxide B (isomer 2, 9.1%), bisabolol oxide B (isomer 1, 7.3%), crassifolone (6.7%), dihydrocrassifolone (5.7%)	[15]
M. deserti A. Cunn.	Leaf (CS_2 extract)	(1*R*)-1-acetoxymyodesert-3-ene (30%), (1*S*)-1-acetoxymyodesert-3-ene (50%)	[16]
M. deserti A. Cunn.	Leaf	Dehydrongaione (90%), isodehydrongaione (4%), dehydroepingaione (6%)	[17]
M. laetum G. Forst.	Leaf	Ngaione (26.0–44.7%), elemicin (16.6–50.2%), dehydromyoporone (0.4–13.5%), β-elemene (0.6–12.2%), germacrene D (0.7–6.0%)	[18]
M. montanum R.Br.	Leaf	Myomontanone (70%), myoporone (22%), isomyomontanone (3%)	[19]
M. montanum R.Br.	Leaf (acetone extract)	Myoporone (16.0%), bicyclogermacrene (10.5%), germacrene D (9.0%), 10,11-dehydroisomyodesmone (5.2%), 10,11-dehydromyodesmone (4.5%)	[20]
M. tetrandrum (Labill.) Domin	Leaf	Dehydrongaione (78%), ngaione (7%), myoporone (11%)	[17]

3.2. Antimicrobial Activity

M. sandwicense essential oils were screened for antimicrobial activity against a panel of potentially pathogenic bacteria and fungi (Table 6). The leaf essential oil of *M. sandwicense* showed excellent antibacterial activity against *S. pyogenes* (MIC = 78 μg/mL) and antifungal activity against *A. fumigatus* (MIC = 39 μg/mL). In fact, these two organisms were the most susceptible in our panel. The major components in the leaf essential oil: β-caryophyllene, α-humulene, bicyclogermacrene, and myoporone, may be responsible for the antimicrobial activity. Both β-caryophyllene and α-humulene have shown antimicrobial activity [21]. In earlier investigation, the essential oil of *Centella asiatica*, also rich in β-caryophyllene, α-humulene, and bicyclogermacrene, showed antibacterial activity [22]. Myoporone has shown antibacterial activity [20,23]. The wood essential oil of *M. sandwicense*, which was dominated by α-bisabolol and α-bisabolol oxide B, also showed notable activity against *S. pyogenes* and *A. fumigatus* (MIC = 78 μg/mL for each) as well as *A. niger* and *M. gypseum* (MIC = 39 μg/mL for each). α-Bisabolol has shown marginal antibacterial activity [24], but good antifungal activity [25,26]. In addition, α-bisabolol has been shown to potentiate the antibacterial activities of several antibiotics [27,28].

Table 6. Antimicrobial activities, MIC [a] (μg/mL), of essential oils of *Myoporum sandwicense*.

Microorganism	Leaf	Wood	Twigs
Gram-positive bacteria			
Bacillus cereus	2500	2500	313
Cutibacterium acnes [b]	156	313	78
Staphylococcus aureus	2500	2500	156
Staphylococcus epidermidis	2500	2500	156
Streptococcus pneumoniae	156	156	1250
Streptococcus pyogenes	78	78	156
Gram-negative bacteria			
Escherichia coli	2500	625	625
Helicobacter pylori	313	313	313
Pseudomonas aeruginosa	2500	2500	313
Salmonella typhimurium	313	313	313
Serratia marcescens	625	625	625
Molds			
Aspergillus fumigatus	39	78	78
Aspergillus niger	156	39	78
Microsporum canis	2500	2500	39
Microsporum gypseum	2500	39	39
Trichophyton mentagrophytes	2500	2500	39
Yeasts			
Candida albicans	2500	2500	156
Cryptococcus neoformans	313	313	313

[a] Minimum inhibitory concentration. [b] Formerly *Propionibacterium acnes*.

The antifungal activity of the essential oil from the twigs against the mold species was surprising. The twig essential oil was composed of 95.4% *n*-alkanes. Yin and co-workers have examined the antifungal activity of cuticular wax from Asian pear fruit (*Pyrus bretchneideri*) and concluded that long-chain alkanes have antifungal activity [29]. The flower SFE-CO2 extract of black elderberry, rich in ethyl palmitate, n-pentacosane, n-tricosane, and n-heneicosane, showed antifungal activity [30]. In contrast, the alkane-rich wood essential oil of agarwood (*Aquilaria sinensis*) showed only marginal antifungal activity [31]. Similarly, *n*-alkane-rich fractions from a hexane extract of *Cupressus lusitanica* leaves (>90% alkanes) showed no antifungal activity against a panel of dermatophytes [32]. It is likely that synergistic interactions with the minor components account for the observed antifungal activity of the twig essential oil. The non-polar fraction of surface wax from Asian pear fruits, dominated by long-chain n-alkanes showed inhibition of germination of *Alternaria alternata* [29]. Likewise, sorghum leaf wax, rich in long-chain alkanes, suppressed the growth of *A. alternata* [33]. Several long-chain n-alkanes have been screened for inhibition of mycelia and conidial germination of *A. alternata* and were found to be inactive [34]. Since alkanes do not have functional groups, they are unlikely to interact with biological targets involved with fungal metabolic pathways (e.g., glyoxylate cycle, pyrimidine biosynthesis, cytochrome P450 enzymes, iron metabolism, heme biosynthesis, and acetate metabolism), signal transduction pathways (e.g., MAP kinase, PDK1, and calcium signaling), or gene expression [35]. They are, however, non-polar compounds and would be expected to interact with fungal membranes and disturb membrane integrity, affecting membrane permeability. Thus, n-alkanes may act synergistically with other components in the essential oil, allowing these components to diffuse into the fungal cells.

4. Conclusions

This work is the first report on the chemical composition and antimicrobial properties of *Myoporum sandwicense* A. Gray (naio) essential oils. The leaf essential oil was made of β-caryophyllene, α-humulene, germacrene D, bicyclogermacrene, brigalow ketol, and myoporone, while the wood essential oil was dominated by α-bisabolol and *trans*-α-bisabolol oxide B. Palmitic acid, linoleic acid, oleic acid, and stearic acid were the major components

of the sapwood oil, whereas the oil from twigs was rich in tricosane and pentacosane. The leaf essential oil of *M. sandwicense* showed excellent antibacterial activity against *Streptococcus pyogenes* and antifungal activity against *A. fumigatus*. The wood essential oil showed notable activity against *S. pyogenes*, *A. fumigatus*, *A. niger*, and *M. gypseum*. The twig oil was remarkably active against mold species.

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