Antimicrobial Constituents of the Leaves of *Mikania micrantha* H. B. K.

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Abstract

**Background:** To isolate plant-derived compounds with antimicrobial activity from the leaves of *Mikania micrantha*, to determine the compounds configuration, and to evaluate their antimicrobial activity against eight plant pathogenic fungi (*Exserohilum turcicum*, *Colletotrichum lagenarium*, *Pseudoperonospora cubensis*, *Botrytis cinerea*, *Rhizoctonia solani*, *Phytophthora parasitica*, *Fusarium solani*, and *Pythium aphanidermatum*) and four plant pathogenic bacteria (gram negative bacteria: *Ralstonia dolaranaearum*, *Xanthomonas oryzae* pv. *Oryzae*, *Xanthomonas campestris* pv. *Vesicatoria*, and *Xanthomonas campestris* pv. *Citri*), and four bacteria (gram positive bacteria: *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, and *Bacillus cereus*).

**Methods and Results:** Antimicrobial constituents of the leaves of *M. micrantha* were isolated using bioactivity-guided fractionation. The antifungal activity of the isolated compounds was evaluated by the inhibit hypha growth method and inhibit spore germination method. Characterization of antibacterial activity was carried out using the minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs). MIC and MBC were determined by the broth microdilution method. Six compounds – deoxymikanolide, scandenolide, dihydrosandenolide, mikanolide, dihydromikanolide, and m-methoxy benzoic acid – have been isolated from leaves of *Mikania micrantha* H. B. K. Deoxymikanolide, scandenolide, and dihydrosandenolide were new compounds. The result of bioassay showed that all of isolated compounds were effective against tested strains and deoxymikanolide showed the strongest activity.

**Conclusions and Significance:** The leaves of *M. micrantha* may be a promising source in the search for new antimicrobial drugs due to its efficacy and the broadest range. Meanwhile, adverse impact of *M. micrantha* will be eliminated.

Introduction

*Mikania micrantha* H. B. K. (Asteraceae), commonly known as mile-a-minute weed, is an extremely fast-growing, perennial creeping weed. Its native distribution is in Central and South America. It has been present in south China since the 1980s and has caused adverse impact on agricultural production in the established area [1]. So, the weed has been listed as one of the world’s 100 worst invasive alien species by Invasive Species Specialist Group of IUCN [2]. The leaves of *M. micrantha*, commonly known as ‘guaco’, are used to make a poultice for snake bites and scorpion sting, decoction of the leaves is used to bathe rashes, and skin itches [3]. In Jamaica, its most popular uses are for wound dressings and promote the healing of sores as folk medicine [4]. In addition, it has been attracted the attention of natural products chemists because of its antibacterial, antitumor, cytotoxic, analgesic, inflammatory, antiproliferative, and phytotoxic activities [5–13]. By consulting literatures, sterols [14], [15], diterpenes [15], polyphenols [16], flavonoids [17–19], and sesquiterpene lactones [4], [14], [20] have been isolated from *M. micrantha*. However, the antimicrobial constituents were ambiguous, though mikanolide and dihydromikanolide as antimicrobial ingredient of *M. micrantha* were reported. With the aim of identifying those potential additional and bioactive natural products of this plant, a phytochemical study has been carried out bioactivity-guided fractionation and resulted in the isolation of three new sesquiterpene lactones, named deoxymikanolide, scandenolide, and dihydrosandenolide [1–3], together with three known compounds [4–6]. Compound 6 was named as m-methoxy benzoic acid, which was the first reported occurrence from this plant. Herein, the isolation and structural elucidation of these compounds, as well as their antimicrobial activities against eight bacteria and eight fungi, were described. This study provides first comprehensive description about the antimicrobial constituent and antimicrobial activities in an attempt to improve the understanding of the practicality of *M. micrantha*. 
### Materials and Methods

#### Plant Material.
The leaves of *M. micrantha* were collected in April 2011, in Dongguan, Guangdong Province of China. The sample was identified by Professor Xiao-yi Wei. An authenticated voucher specimen (No. 020701) was deposited at the herbarium of South China Institute of Botany, Chinese Academy of Sciences, Guangzhou, and People’s Republic of China. We state clearly that no specific permissions were required for these locations, because these locations are uncultivated land. We confirm that the field studies did not involve endangered or protected species.

#### Fungal Strains.
Eight reference fungi, *Exserohilum turci-cum*, *Colletotrichum lagenarium*, *Pseudoperonispora cubensis*, *Botrytis cinerea*, *Rhizoctonia solani*, *Phytophthora parasitica*, *Fusarium solani*, and *Pythium aphanidermatum*, were used during the study. The tested strains were obtained from the Institute of Plant Protection, Chinese Academy of Agricultural Sciences China. The fungi were inoculated in beef extract peptone agar medium (PBA) at 27°C and stored in nutrient agar slants at 4°C.

#### Bacterial Strains.
Eight reference bacteria, *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *Bacillus cereus*, *Ralstonia dolaanaccarum*, *Xanthomonas oryzae pv. Oryzae*, *Xanthomonas campestris pv. Vescatoria*, and *Xanthomonas campestris pv. citri* were used during the study. The tested strains were obtained from the Institute of Plant Protection, Chinese Academy of Agricultural Sciences China. The tested strains were cultured in beef extract peptone agar medium (PBA) at 27°C and stored in nutrient agar slants at 4°C.

#### Extraction and isolation
The air-dried leaves powder of *M. micrantha* (2.0 kg) was ultrasonic extracted with chloroform (6L×3) at ambient temperature. Activated carbon was added in the extract for decolorization, then filtered in order to get rid of activated carbon and undissolved substance. The extract was concentrated in vacuo to give 122.60 g of the crude residue (yield 6.13%). The crude residue (120 g) was subjected to chromatography on silica gel (100–200 mesh) eluting successively with gradient carbon tetrachloride – acetone – methanol (35:2:1, 35:5:1, 35:5:2 35:6:2, 35:6:3, 35:6:4, 35:6:5, v/v) to give fractions A-1 to A-30. A-11 (1.8434 g) was rechromatographed on a silica gel column eluted with chloroform: ethyl acetate (4:1 v/v) followed by crystallization from chloroform to give compound 1 (0.8310 g 0.04155% yield) and compound 2 (0.3512 g 0.01756% yield). A-14 (1.2306 g) was rechromatographed on a silica gel column eluted with chloroform: acetone – methanol (35:2:1, 35:5:1, 35:5:2 35:6:2, 35:6:3, 35:6:4, 35:6:5, v/v) to give fractions A-1 to A-30. A-11 (1.8434 g) was rechromatographed on a silica gel column eluted with chloroform: ethyl acetate (4:1 v/v) followed by crystallization from chloroform to give compound 1 (0.8310 g 0.04155% yield) and compound 2 (0.3512 g 0.01756% yield). A-14 (1.2306 g) was rechromatographed on a silica gel column eluted with petroleum ether: ethyl acetate (5:1 v/v) followed by crystallization from chloroform to give compound 3 (0.4432 g 0.02216% yield). A-16 (5.7534 g) was rechromatographed on a silica gel column eluted with petroleum ether: ethyl acetate (1:1 v/v) followed by crystallization from acetone to give compound 4 (2.7364 g 0.13682% yield). A-17 (2.5618 g) was rechromatographed on a silica gel column eluted with chloroform: ethyl acetate (1:1 v/v) followed by crystallization from acetone to give compound 5 (0.8041 g 0.040205% yield). A-

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### Table 1. ¹H NMR(400 MHz) and ¹³C NMR (300 MHz) spectral data of compound 1–6 (DMSO) (J in Hz).

| No. | ¹H NMR | ¹³C NMR | ¹H NMR | ¹³C NMR | ¹H NMR | ¹³C NMR | ¹H NMR | ¹³C NMR | ¹H NMR | ¹³C NMR | ¹H NMR |
|-----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 1   | 61.16  | 2.86 d | (9.6)  | 57.41  | 3.00 d | (10.1) | 58.41  | 2.98 d | (10.0) | 57.15  | 3.26 s  |
| 2   | 23.19  | 1.61 m | 28.59  | 1.62 m | 29.02  | 1.72 m | 55.25  | 3.38 d | (3.2)  | 56.08  | 3.37 d | (2.8)  |
| 3   | 21.66  | 2.78 m | 66.13  | 5.58 s | 67.09  | 5.60 d | (1.0)  | 49.14  | 4.79 m | 51.54  | 4.02 m  | 130.69 |

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References

1. Xiao-yi Wei, et al. (2014). *Antimicrobial Constituents of Mikania micrantha*.

DOI: 10.1371/journal.pone.0076725.t001
19 (1.7658 g) was rechromatographed on a silica gel column eluted with petroleum ether: ethyl acetate: acetone (15:2.5: v/v/v) followed by crystallization from chloroform to give compound 6 (0.4355 g 0.021775% yield).

Deoxymikanolide (1)

Needle crystals; mp 180–185°C; [α] +57.5 (c 0.4, CH_{2}Cl_{2}); UV (CH_{2}Cl_{2}) λ_{max}; 281, 276 nm. IR (KBr) ν_{max}; 3435, 2924, 2853, 1757, 1658, 1288, 1154, 1044, 464 cm⁻¹. For ¹H and ¹C NMR spectroscopic data, see Table 1. Positive HRESIMS m/z 277.0294 [M+H]⁺ (calc. for C_{15}H_{17}O_{5}NaH, 334.3100). The Mass spectral data of deoxymikanolide were 277.0294, 269.0351, 253.06253, and 242.028345.

Scandenolide (2)

Needle crystals; mp 160–165°C; [α] +43.2 (c 0.5, CH_{2}Cl_{2}); UV (CH_{2}Cl_{2}) λ_{max}; 262, 273 nm. IR (KBr) ν_{max}; 3435, 2924, 2853, 1770, 1659, 1369, 1284, 1293, 685 cm⁻¹. For ¹H and ¹C NMR spectroscopic data, see Table 1. Positive HRESIMS m/z 357.2945 [M+Na]⁺ (calcld for C_{17}H_{18}O_{7}Na, 357.3161). The Mass spectral data of scandenolide were 357.2945, 338.3420, 321.0502, 301.1412, 280.2635, 253.0630, and 238.0146. Upon crystallization from CHCl₃ using the vapor diffusion method, colorless needles of compound 2 were obtained. A suitable crystal was selected and performed on a SuperNova, Dual, and Cu at zero, Atlas diffractometer. The crystal was kept at 180.00(10) K during data collection. Using Olex2 [21], the structure was solved with the Superflip [23] structure solution program using Charge Flipping and refined with the XL [22] refinement package using Least Squares minimisation. Crystal data: C_{17}H_{20}O_{7}, M = 316.33, space group monoclinic, P2₁; unit cell dimensions were determined to be a = 9.2297 (6) Å, b = 6.0316 (5) Å, c = 14.6466 (13) Å, β = 90.00° ; γ = 90.00°; V = 912.12 (14) Å³, Z = 2, Dcalc = 1.369 mg/mm³, crystal size 0.2×0.1×0.05 mm, μ (Mo Kα) = 0.1091 mm⁻¹, F(000) = 556.0, T = 296.15(10) K, 12798 reflections measured (6.54 ≤ 2θ ≤ 52.04), 2719 unique (Rint = 0.0291) which were used in all calculations. The final R₁ = 0.0362 (>2σ(I)) and wR₂ was 0.0854. In the structure refinements, nonhydrogen atoms were placed on the geometrical ideal positions by the “ride on” method. Hydrogen atoms bonded to oxygen were located by the structure factors with isotropic temperature factors. Crystallographic data for 2 have been deposited at the Cambridge Crystallographic Data Centre (deposition No. CCDC- 922241).

Dihydrosandenolide (3)

Needle crystals; mp 270–275°C; [α] +54.3 (c 0.1, CH_{2}Cl_{2}); UV (CH_{2}Cl_{2}) λ_{max}; 257, 288 nm. IR (KBr) ν_{max}; 3421, 2921, 1750, 1639, 1233, 1154, 1034, 675 cm⁻¹. For ¹H and ¹C NMR spectroscopic data, see Table 1. Positive HRESIMS m/z 337.0241 [M+H]⁺ (calc. for C_{15}H_{16}O_{5}NaH, 337.3502). The Mass spectral data of dihydrosandenolide were 337.0241, 321.0514, 305.0736, 286.0263, and 270.0535.

Single crystal X-ray diffraction analysis of 1, 2, and 3

Upon crystallization from CHCl₃-EtOAc (1:1) using the vapor diffusion method, colorless needles of compound 1 were obtained. A suitable crystal was selected and performed on a SuperNova, Dual, and Cu at zero, Atlas diffractometer. The crystal was kept at 180.00(10) K during data collection. Using Olex2 [21], the structure was solved with the Superflip [23] structure solution program using Charge Flipping and refined with the XL [22] refinement package using Least Squares minimisation. Crystal data: C_{17}H₂₀O_{7}, M = 336.33, space group monoclinic, P2₁; unit cell dimensions were determined to be a = 6.2346 (3) Å, b = 9.5598 (5) Å, c = 13.4299 (7) Å, α = 90.00°; β = 91.687 (5); γ = 90.00°; V = 800.09 (7) Å³, Z = 2, Dcalc = 1.396 mg/mm³, crystal size 0.2×0.1×0.05 mm, μ (Mo Kα) = 0.1091 mm⁻¹, F(000) = 356.0, T = 180.00(10) K, 4879 reflections measured (6.54 ≤ 2θ ≤ 52.04), 2719 unique (Rint = 0.0221) which were used in all calculations. The final R₁ = 0.0362 (>2σ(I)) and wR₂ was 0.0854. In the structure refinements, nonhydrogen atoms were placed on the geometrical ideal positions by the “ride on” method. Hydrogen atoms bonded to oxygen were located by the structure factors with isotropic temperature factors. Crystallographic data for 3 have been deposited at the Cambridge Crystallographic Data Centre (deposition No. CCDC- 939629).

Antibacterial activity assay

MIC and MBC of the compounds were assessed using the broth microdilution method recommended by the National Committee for Clinical Laboratory Standards [24], [25]. Inoculums of the microorganisms were prepared from 24 h Mueller-Hinton Broth (MHB) cultures and suspensions were adjusted with turbidity equivalent to that of a 0.5 McFarland standard. The compounds dissolved in 5% dimethylsulfoxide (DMSO), were first diluted to the highest concentration (2000 mg/L) to be tested, and then serial twofold dilutions were made in a concentration range from 15,625 to 1000.0 mg/L in 5 ml sterile test tubes containing the medium. The 96-well plates were prepared by dispensing into each well 95 µL of the medium without the compounds and 5 µL of the inoculum. A 100 µL aliquot from the stock solutions of each isolates was added into the first wells. Then, 100 µL from the serial dilutions were transferred into 6 consecutive wells. The final well containing 195 µL of the medium without the compounds and 3 µL of the inoculum on each strip was used as negative control. The final volume in each well was 200 µL. Broth with 5 µL of DMSO was used as blank test. Plates were covered and incubated for 12 h at 37°C. After incubation, the lowest concentration of tested samples, which did not show any visual growth after macroscopic evaluation, was determined as MIC. Using the results of the MIC assay, the concentrations showing complete absence of visual growth of bacteria were identified and 10 µL of each culture broth was transferred on to the agar plates and incubated for the specified time and temperature as mentioned above. The complete
absence of growth on the agar surface in the lowest concentration of sample was defined as the MBC. All tests were performed in triplicate.

**Antifungal activity assay**

Antifungal activities of the compounds was tested using the inhibit hypha growth method. The compounds were prepared with different concentrations using dimethyl sulfoxide (DMSO) and diluted by Potato Dextrose Agar (PDA) medium as 500, 250, 125, 62.5, and 31.25 mg/L. The PDA mediums mixed with the compounds were dumped respectively into petri dishes (9 cm diameter) as plating. An agar plug of fungal inoculums (6 mm in diameter) was removed from a previous culture of the fungal strains tested and placed upside down in the center of the petri dishes. The same amount of DMSO and distilled water which

![Figure 1. Structures of compounds from the leaves of *Mikania micrantha*. Solid bonds indicate carbon chain. Wedge bonds indicate forwardly chemical bond. Hashed wedged bonds indicate rearwards chemical bond.](doi:10.1371/journal.pone.0076725.g001)

![Figure 2. Selected $^1$H-$^1$H COSY and HMBC corrections for deoxymikanolide. COSY means correlated spectroscopy and HMBC means ($^1$H-detected) heteronuclear multiple-bond correlation. Bold bond indicate the correlation of hydrogen atoms of adjacent carbon. Arrowed line indicates that hydrogen atom correlate with neighboring carbon (arrow for the carbon atom).](doi:10.1371/journal.pone.0076725.g002)

![Figure 3. Selected NOESY correlations and relative configuration for deoxymikanolide. NOESY means nuclear Overhauser effect spectroscopy. Dashed bonds indicate the correlation of hydrogen atoms of carbon atoms in two-dimensional space.](doi:10.1371/journal.pone.0076725.g003)
were used to replace the compounds were added respectively into the PDA mediums as the blank test and negative control. Each treatment was done with three replicates. All of materials were subjected to autoclaving at 121°C for 30 min. The means diameter of fungal colony was measured by criss-cross method with calipers by incubated at 28°C.

Inhibit spore germination activity assay

The spore suspensions of Botrytis cinerea, Glomerella cingulata, Exserohilum turcicum, Fusarium solani were obtained from their respective 10 days old cultures. The spore suspension was adjusted with sterile distilled water to a concentration of approximately $1.0\times10^5$ spore/mL. The compounds dissolved in 5% dimethyl sulfoxide (DMSO), and then serial twofold dilutions with the spore suspensions were made in a concentration range from 125.0 to 2000.0 mg/L. 100 μL of the spore suspensions were taken onto the glass slide and incubated at 24±2°C for 24 h. About 200 spores were counted and percentage of spore germination was calculated. The same amount of DMSO and distilled water were used as negative control and blank test. All experiments were conducted in triplicate. All of materials were subjected to autoclaving at 121°C for 30 min.

Statistical analysis

Data were analyzed using SPSS 10.0 (Chicago, IL, USA). The data were considered statistically significant for P values <0.05.

Results

Compound 1–6 were isolated from the leaves of M. micrantha using bioactivity-guided fractionation and their structures were showed in Figure 1.
Compound 1 was obtained as colorless needle crystals in chloroform, and gave \([\text{M+H}]^+\) peak at \(m/z\) 277.0294 (calc. for \(\text{C}_{15}\text{H}_{16}\text{O}_5\)) in HR-ESI-MS, corresponding to the molecular formula \(\text{C}_{15}\text{H}_{16}\text{O}_5\), indicating eight degrees of unsaturation. Compared with the spectral data of the sesquiterpene dilactone isolated from this plant, its 1D NMR spectroscopic data were suggestive of the presence of a special guaianolide skeleton (Table 1). In the \(1^H-1^H\) COSY experiments, the correlations of \(H-1\) through \(H-2\) to \(H-3\), \(H-5\) through \(H-6\), \(H-7\), \(H-8\), and \(H-9\), to \(H-10\), \(H-7\) to \(H-13\) established two fragments. The HMBC correlations between olefinic proton \(H-5/C-4\), \(C-6\), and \(C-15\) suggested the presence of cyclic olefinic lactone functionality. Moreover, the observed correlation of olefinic group \((C-11\) and \(C-13\)), the ester carbon \((C-12)\), and exocyclic olefinic protons \((H-13a\) and \(13b)\) authenticated the existence of characteristic methylene lactone functionality (Figure 2). On the basis of above data and compared with the spectral data of known sesquiterpenes dilactones isolated from this plant, compound 1 was identified as deoxymikanolide [19], [26]. But its relative stereochemistry have never been reported, which these papers only listed \(^{13}\text{C}\) and \(^1\text{H}\) NMR spectral data. The relative stereochemistry of 1 was further confirmed by detailed analysis of NOESY spectra (Figure 3) and single-crystal-X-ray diffraction study (Figure 4). In the NOESY spectrum, the correlations of \(H-1/H-8\), and \(H-6/H-1\) indicated that \(H-1\), \(H-6\), and \(H-8\) adopted the same orientation and were arbitrarily designated as the \(\beta\)-orientation. The NOESY correlations of \(H-14/H-7\) revealed that Me-14 and \(H-7\) were \(\alpha\)-orientation (Figure 3). Its configuration as established by NOESY spectrum was in good agreement with the X-ray diffraction study. A single crystal of 1 was obtained and subjected to X-ray crystallographic diffraction analysis. The result unambiguously confirmed the structure of 1 and the relative stereochemistry of 1 was determined to be \(1R, 6R, 7R, 8S,\) and \(10S\). Consequently, compound 1 was elucidated as \(1,10\)-epoxy-6,8-dihydroxy-11-vinylgermacr-4-ene12, 14-di-\(\gamma\)-lactone and \(7,10\text{a}\)-dimethyl-1a,1b,2a,6a,7,9a,10,10a-octahydro-\(\text{H}-6,3\)-methenofuro[3,2-c]...
biosoxycloundecin-4,8(H)-dione, trivially named deoxymikanolide (Figure 1).

Compound 2 was obtained as colorless needle crystals in chloroform, and gave [M+Na]+ peak at m/z 357.2945 (calc. for C_{17}H_{18}O_{7}Na, 357.3161) in HR-ESI-MS, corresponding to the molecular formula C_{17}H_{18}O_{7}Na, indicating nine degrees of unsaturation. Just as compound 1, its 1D NMR spectroscopic data were suggestive of the presence of a special guaianolide skeleton too (Table 1). Just as compound 1, relative stereochemistry of scandenolide was determined to be 1R, 3S, 6R, 7R, 8S, and 10S. Consequently, compound 2 was elucidated as 1,10-dimethyl-3,6-dioxo-2,6-dihydroxyno[3,2-c]oxireno[2,3-f][1]oxacycloundecin-4,8(H)-dione, trivially named dihydrosandenolide (Figure 1).

Compounds 3-6 (Figure 1) were, respectively, identified as mikanolide, dihydromikanolide [19], m - methoxy benzoic acid from this plant. Just as compound 1 and 2, the relative stereochemistry of dihydrosandenolide was not clear. The relative stereochemistry of 3 was further confirmed by detailed analysis of 1H-1H COSY, HMBC data (Figure 8), and NOESY spectra (Figure 9) and a single-crystal-X-ray diffraction study (Figure 10). In the NOESY spectrum, the correlations of H-13/H-14 revealed that Me-14 and C-13 were β-orientation (Figure 9). As shown in Table 1, all 6 compounds were subjected to quantitative bioassays for antimicrobial activity. DMSO used at 2.5% (v/v) showed no antimicrobial activity. DMSO used at 2.5% (v/v) showed no antimicrobial activity against all tested microorganisms (data not shown). All 6 compounds were subjected to quantitative bioassays for antimicrobial activity. DMSO used at 2.5% (v/v) showed no antimicrobial activity against all tested microorganisms (data not shown).

Figure 10. Single-crystal X-ray structure of dihydrosandenolide. As shown in figure 4.

doi:10.1371/journal.pone.0076725.g010

As shown in Table 2, 3, and 4, all isolates were found to show significantly more antibacterial activity compared with the others against tested bacteria grown in suspension; the MIC values for tested strains (n = 8) ranged from 62.5 to 125 and the MBC values ranged from 125 to 250 (Table 2). The others...
were showed moderate activity against all tested bacteria. By the inhibit hypha growth method, it was seen that EC 50 values of 6 isolated for tested fungi (n = 4) ranged from 82.40 to 330.10 mg/L in different times (Table 3). It is observed that antifungal susceptibility of 6 isolated was not significantly different. The results of inhibit spore germination assay showed that 1 was observed the highest inhibition activity; IC 50 values ranged from 21.44 to 53.18 mg/L (Table 4). Compound 6 was weakest in activity.

Discussion

In recent decade, problems of multidrug resistant microbes have reached an alarming level in around the world. These pose a serious challenge to the scientific community hence emphasis has been laid on the development of new antimicrobial agents [28], [29]. One possible strategy is the rational localization of bioactive products from folk medicines, with the hope that systematic screening of these will result in the discovery of novel effective

Table 3. Antifungal activity of compound 1–6 against tested fungi strains.

| Tested Fungus Strains | Time (h) | EC 50 (mg L⁻¹) |
|-----------------------|----------|----------------|
|                        | 1        | 2              | 3              | 4              | 5              | 6              |
| Fusarium solani        | 48       | 82.40          | 101.42         | 100.23         | 107.91         | 100.23         | 95.28          |
|                        | 72       | 97.68          | 126.27         | 113.07         | 110.25         | 113.07         | 116.15         |
|                        | 96       | 116.74         | 144.51         | 121.38         | 117.96         | 121.38         | 122.96         |
| Rhizoctonia solani     | 48       | 109.59         | 117.60         | 132.29         | 138.35         | 132.29         | 135.04         |
|                        | 72       | 157.44         | 169.32         | 174.17         | 197.16         | 174.17         | 194.32         |
|                        | 96       | 263.64         | 282.98         | 290.77         | 329.19         | 290.77         | 310.00         |
| Pythium aphanidermatum | 48       | 114.43         | 153.20         | 212.90         | 205.88         | 212.90         | 217.63         |
|                        | 72       | 129.12         | 222.83         | 279.25         | 254.78         | 279.25         | 273.80         |
|                        | 96       | 167.26         | 254.34         | 347.33         | 288.15         | 347.33         | 330.10         |
| Phytophthora parasitica| 48       | 116.17         | 161.10         | 145.03         | 149.22         | 145.03         | 249.86         |
|                        | 72       | 125.53         | 169.88         | 166.72         | 159.47         | 166.72         | 278.10         |
|                        | 96       | 156.73         | 166.53         | 205.12         | 153.16         | 205.12         | 316.41         |

Table 4. Inhibition of spore germination activity of compound 1–6 against tested fungi strains.

| Tested Fungus Strains | IC 50 (mg L⁻¹) |
|-----------------------|---------------|
|                        | 1              | 2              | 3              | 4              | 5              | 6              |
| Exserohilum turcicum   | 21.44          | 86.99          | 93.42          | 80.41          | 76.04          | 255.37         |
| Botrytis cinerea       | 36.53          | 113.08         | 77.55          | 86.40          | 62.33          | 123.51         |
| Pseudoperonispora cubensis | 53.18       | 75.18          | 79.38          | 132.19         | 67.78          | 211.24         |
| Colletotrichum lanigerum | 40.56       | 110.97         | 64.58          | 80.36          | 81.34          | 167.68         |

doi:10.1371/journal.pone.0076725.t003
doi:10.1371/journal.pone.0076725.t004
compounds with potent and useful activities against microbes. Now, there is an ever-increasing demand for plant-based therapeutics in both developing and developed countries due to a growing recognition that they are natural products, non-narcotic and, in most cases, easily available at affordable prices; they also have no side effects.

In China, since its introduction in the late 1980s and the early 1990s, *M. micrantha* has drastically infested Guangdong Province and caused significant damage to forests, farmlands, and orchards; and led to great loss of native species diversity, significant decline of microbial communities and food web stability; and even altered mineral cycling [30]. Though, *M. micrantha* was used in the traditional pharmacopoeia in some countries. So, the aims of this study not only develop to new antimicrobial agents, but also providing theoretical basis for the rational exploiting and controlling of *M. micrantha*.

Early reports on *M. micrantha* revealed its antimicrobial action; mikanolide and dihydromikanolide were major antimicrobial activity constituents [4]. In the present study, antimicrobial activity compounds were further isolated and the 6 compounds were obtained using bioactivity-guided fractionation, including 3 new sesquiterpene dilactones - deoxymikanolide, scandelonide, and dihydroscedenolide, and 3 known isolates - mikanolide, dihydromikanolide, and m - methoxy benzoic acid. Structure elucidation of 3 new sesquiterpene dilactones were determined according to 1D, 2D NMR, IR, and single-crystal-X-ray diffraction study. In order to reveal effective substances for bioactivities in vitro, 6 isolates were tested for antibacterial activities against four gram-negative and four gram-positive bacteria by the broth microdilution methods. The obtained MIC and MBC values ranged from 62.5 to 1000 mg/L, especially, deoxymikanolide possessed the highest antimicrobial activity; the others only demonstrated moderate antimicrobial activities. Antifungal activities of 6 isolates were described against eight plant pathogens fungi. The results showed that EC$_{50}$ values ranged from 82.40 to 530.10 mg/L in different times by the inhibit hypha growth method and IC$_{50}$ values ranged from 21.44 to 253.37 mg/L by inhibit spore germination method. This study showed that antimicrobial constituents of *M. micrantha* were separated. In addition, structures of five compounds possess special guaianolide skeleton, familiar structure of the compounds were reported in *Mikania* species [8], [31–33]. So, the skeleton as parent structure should synthesis and structure-function relationship should further study. Occurrences of dilactone in this special guaianolide skeleton suggest that the problem of water-solubility of these active compounds will be solved by hydrolysis reaction, but bioactivity of the compounds will be tested in future experiment.

The results further demonstrated the broadest range of antimicrobial properties of *M. micrantha*. Considering the broadest range of antimicrobial properties, this would suggest that *M. micrantha* might be useful as a broad-spectrum antimicrobial. The mechanism of action will be done in further study on the basis of in vitro experimentation for development of new products. The isolation and bioactivity results will provide scientific foundation for rational development and utilization of this plant. If possible from collected specifically for controlling pathogen agent use, in order to manage indirectly and reduce adverse impact about *M. micrantha*.

**Author Contributions**

Conceived and designed the experiments: Yan Li AC. Performed the experiments: Yan Li JL. Yuan Li. Analyzed the data: Yan Li JL. Contributed reagents/materials/analysis tools: Yan Li XW. Wrote the paper: Yan Li.

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