

Article

Sesquiterpenoids from the Inflorescence of *Ambrosia artemisiifolia*

Zhi Zeng^{1,†}, Hong Huang^{2,†}, Hualiang He¹, Lin Qiu¹, Qiao Gao¹, Youzhi Li^{1,3} and Wenbing Ding^{1,3,*}¹ College of Plant Protection, Hunan Agricultural University, Changsha 410128, China² Institute of Plant Protection, Hunan Province, Changsha 410125, China³ Hunan Provincial Engineering & Technology Research Center for Biopesticide and Formulation Processing, Changsha 410128, China

* Correspondence: dingwenb119@hunau.edu.cn

† These authors contributed equally to this work.

Abstract: The successful invasion of *Ambrosia artemisiifolia* is largely due to allelopathy. As an invasive alien plant, *A. artemisiifolia* has spread rapidly in Asia and Europe. Studies have shown that sesquiterpenoids play an important role in plant allelopathy. However, it is unclear whether the inflorescence of *A. artemisiifolia* also contains allelopathic components. In this paper, our phytochemical research focuses on the inflorescence of *A. artemisiifolia*. Twenty sesquiterpenoids, including four new ones (1–4) were isolated through successive chromatographic columns and identified by spectroscopic methods. At a concentration of 200 µg/mL, all the compounds tested were evaluated for their allelopathic activities on seedling growth of wheat. Our results indicate that nine compounds inhibited both the root and shoot growth of seedlings. Compounds 14, 15, 17, and 20 significantly inhibited root length, which was more than 50% shorter than the control. This study identified the chemical profile of the sesquiterpenoids occurring in the inflorescence of *A. artemisiifolia*. The bioactivity screening results provide further understanding of the chemical basis of allelopathy in *A. artemisiifolia*.

Keywords: *Ambrosia artemisiifolia*; sesquiterpenoids; allelopathy activity

Citation: Zeng, Z.; Huang, H.; He, H.; Qiu, L.; Gao, Q.; Li, Y.; Ding, W. Sesquiterpenoids from the Inflorescence of *Ambrosia artemisiifolia*. *Molecules* **2022**, *27*, 5915. <https://doi.org/10.3390/molecules27185915>

Academic Editor: Valeria Patricia Stilsen

Received: 8 August 2022

Accepted: 9 September 2022

Published: 12 September 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Ambrosia artemisiifolia L. (Asteraceae), which is native to North America, is an invasive weed widely distributed throughout temperate regions of the world [1]. This invasive plant has the potential to destroy native ecosystems and reduce agricultural yields [2]. Allelopathy describes the chemicals exuded from roots, rhizomes, leaves, stems, and/or litter of an individual and the resultant beneficial or deleterious effects on other organisms and is an important factor in the successful invasion of alien plants [3]. Meanwhile, because allelopathic components generally have strong phytotoxic activities against many weeds, the allelochemicals have the potential to develop new herbicides, which have the advantages of safety, easy degradation, and no resistance in weed control [4]. Previous studies reported that different solvent extracts of *A. artemisiifolia* had significant inhibitory effects on crop growth and seed germination, weeds, and other plants [5,6]. Studies have shown that sesquiterpenoids are the main chemical components of *Ambrosia* and play an important role in plant allelopathy [7–10]. Every year between July and August, clusters of columnar inflorescence from *A. artemisiifolia* produce large amounts of pollen causing a series of allergic reactions and affecting human health [11]. However, it is unclear whether the inflorescence of *A. artemisiifolia* also contains allelopathic components. Therefore, our phytochemical research focuses primarily on the inflorescence of *A. artemisiifolia*, from which we obtained twenty sesquiterpenoids, including four new ones (1–4). Moreover, all the compounds were evaluated for their allelopathic activities on the seedling growth of wheat.

2. Results and Discussions

Air-dried and powdered inflorescence of *A. artemisiifolia* was extracted with petroleum ether and then further extracted with ethanol. A total of 20 sesquiterpenoids (Figure 1), including four new ones (1–4), were isolated from the petroleum ether extract and ethanol extracts using chromatographic methods.

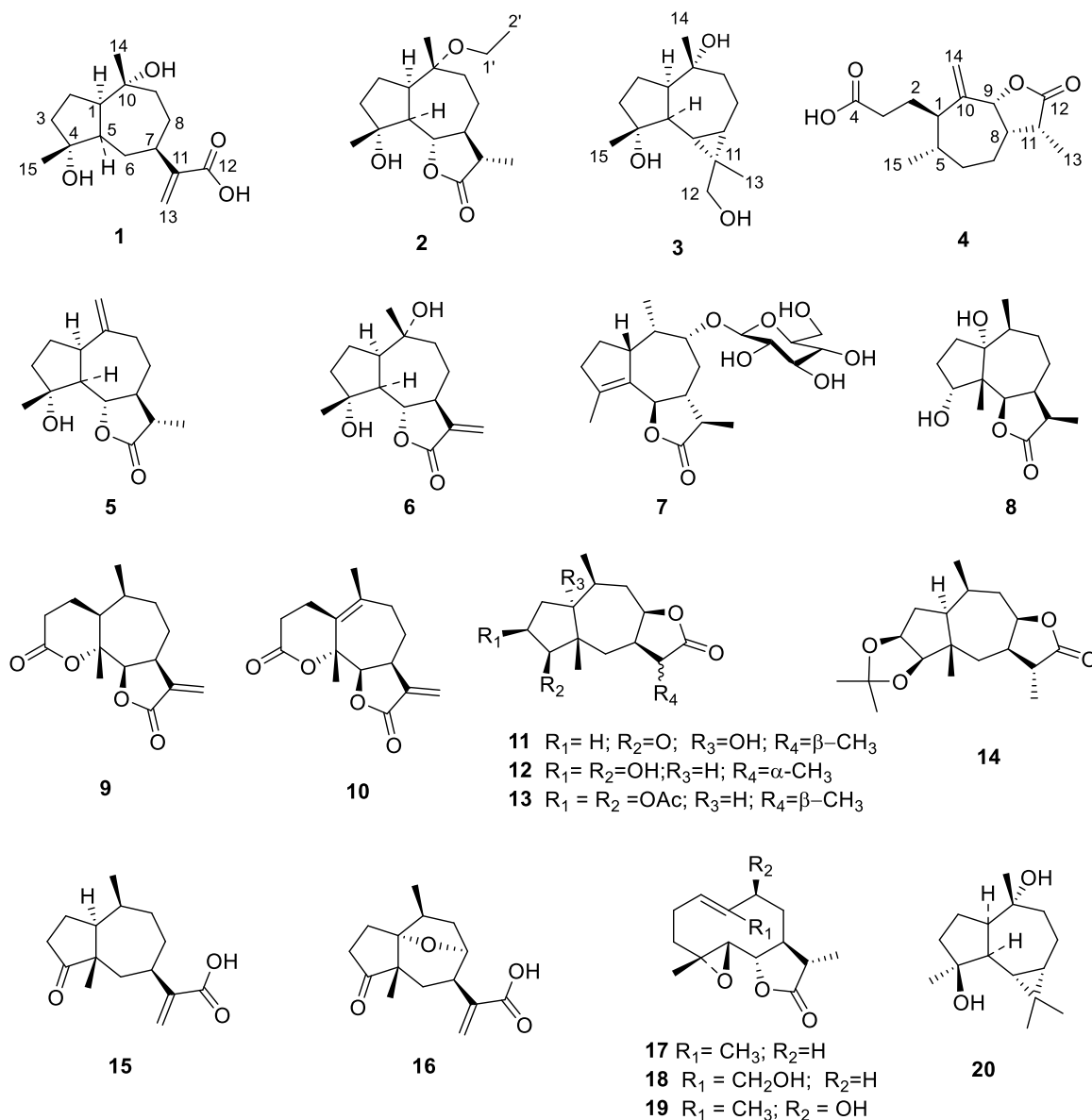


Figure 1. Structures of compounds 1–20 isolated from *A. artemisiifolia*.

2.1. Chemical Characterization of the Compounds

Compound 1 was obtained as a yellowish syrup. The molecular formula was $C_{15}H_{24}O_4$ as determined by the HRESIMS peak at m/z 267.1598 ($[M - H]^-$, calcd. for 267.1596), with four degrees of unsaturation. The 1H NMR data of 1 (Table 1) show two singlet methyls at δ_H 1.50 (H₃-14) and 1.44 (H₃-15) and a terminal alkyne at δ_H 6.48 and 5.68 (H₂-13). The ^{13}C NMR (Table 2) indicated 15 resonances ascribed to two methyls, a terminal alkyne, five methylene, three methines, two oxygenated tertiary carbons, and one α,β -unsaturated carboxylic carbon. The 1H - 1H COSY correlations enabled the establishment of a long coupling carbon chain from C-3 to C-1 that extended via C-5 sequentially to C-9 as shown in bold bonds (Figure 2). The HMBC correlations from H₃-15 (δ_H 1.44) to C-3 (δ_C 40.8), C-4

(80.8), and C-5 (53.8) and from H₃-14 (δ_{H} 1.50) to C-1 (δ_{C} 53.1), C-9 (41.7), and C-10 (73.9) established **1** as a 4,10-dihydroxyguaiane-type sesquiterpene (Figure 2). While HMBC correlations from H₂-13 (δ_{H} 6.48 and 5.68) to C-11 (δ_{C} 150.3), C-12 (170.1), and C-7 (41.6) indicated that a conjugated acrylic acid moiety was attached at C-7. Accordingly, the planar structure of compound **1** was established as 4,10-dihydroxyguaia-11(13)-en-12-acid. The strong NOESY correlation between H-1 and H-5 indicated *cis*-fused framework between the five-carbon ring and the seven-carbon ring. NOEs, H-5 with H-7 show that all of those protons (H-1, H-5, and H-7) were cofacial and were assigned to be α -oriented [12]. In addition, NOEs, H₃-14 with H₃-15 and H₃-15 with H β -6 were observed in the *cis*-fused framework suggesting CH₃-14 and CH₃-15 were β -oriented (Figure 2). Thus compound **1** was determined to be 4 β ,10 β -dihydroxy-1 α ,5 α ,7 α H-guaia-11(13)-en-12-acid.

Table 1. ¹H NMR spectrum (600 MHz) data of compounds 1–4.

| NO. | 1 | 2 | 3 | 4 |
|-----|--|---------------------------------------|--------------------------------------|--|
| | δ_{H} , mult (J in Hz) | δ_{H} , mult (J in Hz) | δ_{H} , mult (J in Hz) | δ_{H} , mult (J in Hz) |
| 1 | 3.14, td (10.3, 8.6) | 2.79, ddd (12.1, 10.7, 8.1) | 1.90, m 1.79, m | 2.21, m / |
| 2 | 2.16, m 1.82, m | 1.84, m 1.55, m | 2.05, m | 2.12, dddd (14.0, 10.1, 8.6, 5.5) 1.90, m |
| 3 | 2.05, dt (12.0, 8.8) 1.90, ddd (11.8, 8.5, 2.7) | 1.76, m 1.77, m | / | 2.58, ddd (15.8, 8.8, 5.5) 2.49, ddd (15.7, 8.6, 7.0) |
| 4 | / | / | 2.56, dd (9.5, 4.4) | / |
| 5 | 2.69, ddd (13.4, 9.9, 3.6) | 2.23, t (12.1) | 0.44, t (9.5) | 1.88, m |
| 6 | 2.19, m 1.79, m | 4.23, dd (11.6, 10.1) | 0.91, ddd (12.1, 9.5, 5.6) | 1.52, m 1.36, m |
| 7 | 3.33, td (10.8, 3.9) / | 1.81, m | 2.26, m 1.93, m | 1.15, m 1.01, td (13.3, 11.5) |
| 8 | 2.22, m 1.80, m | 1.96, m 1.32, m | 1.89, m | 2.40, m |
| 9 | 2.26, m 1.96, ddd (12.6, 9.5, 3.7) | 1.94, m 1.63, td (14.0, 13.4, 4.6) | / | 4.92, m |
| 10 | / | / | 3.36, ddd (12.3, 7.2, 4.5) | / |
| 11 | / | 2.20, m | / | 2.90, m |
| 12 | / | / | 4.15, d (11.1) 4.06, d (11.1) | / |
| 13 | 6.48, d (1.6) 5.68, d (1.6) | 1.22, d (7.0) | 1.42, s | 1.11, d (7.4) |
| 14 | 1.50, s | 1.15, s | 1.43, s | 5.64, d (1.5) 4.93 d, (1.5) |
| 15 | 1.44, s | 1.33, s | 1.73, s | 0.75, d (7.0) |
| 1' | | 3.37, m | | |
| 2' | | 1.11, t (7.0) | | |

Compounds **1**, **3**, and **4** were measured in C₅D₅N, and **2** was measured in CDCl₃.

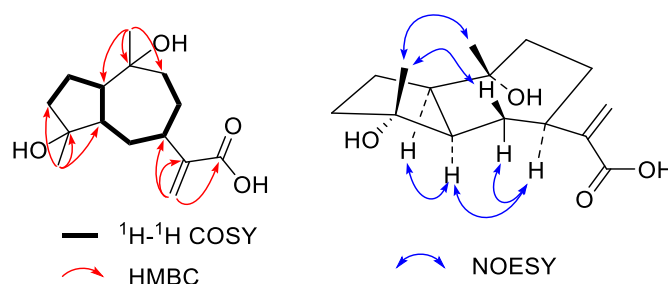


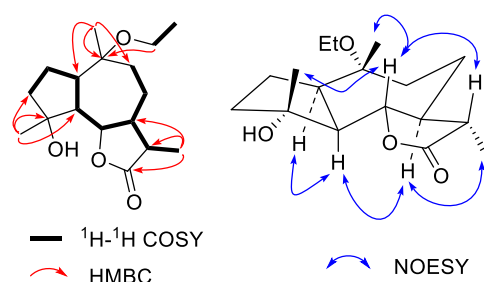
Figure 2. Key ¹H-¹H COSY, HMBC, and NOESY correlations of **1**.

Table 2. ^{13}C NMR spectrum (150 MHz) data of compounds 1–4.

| NO. | 1 | | 2 | | 3 | | 4 | |
|-----|------------|-----------------|------------|-----------------|------------|-----------------|------------|-----------------|
| | δ_c | DEPT | δ_c | DEPT | δ_c | DEPT | δ_c | DEPT |
| 1 | 53.1 | CH | 46.5 | CH | 25.7 | CH ₂ | 43.9 | CH |
| 2 | 26.5 | CH ₂ | 25.6 | CH ₂ | 38.4 | CH ₂ | 29.2 | CH ₂ |
| 3 | 40.8 | CH ₂ | 39.3 | CH ₂ | 81.2 | C | 33.0 | CH ₂ |
| 4 | 80.8 | C | 80.3 | C | 47.8 | CH | 176.2 | C |
| 5 | 53.8 | CH | 55.1 | CH | 26.7 | CH | 34.7 | CH |
| 6 | 33.2 | CH ₂ | 83.0 | CH | 29.4 | CH | 37.4 | CH ₂ |
| 7 | 41.6 | CH | 51.1 | CH | 19.5 | CH ₂ | 18.5 | CH ₂ |
| 8 | 31.7 | CH ₂ | 25.9 | CH ₂ | 39.2 | CH ₂ | 44.8 | CH |
| 9 | 41.7 | CH ₂ | 37.9 | CH ₂ | 73.2 | C | 83.1 | CH |
| 10 | 73.9 | C | 78.0 | C | 54.5 | CH | 143.9 | C |
| 11 | 150.3 | C | 41.4 | CH | 24.9 | C | 39.7 | CH |
| 12 | 170.1 | C | 178.0 | C | 63.0 | CH ₂ | 178.6 | C |
| 13 | 121.4 | CH ₂ | 12.9 | CH ₃ | 24.7 | CH ₃ | 10.9 | CH ₃ |
| 14 | 28.0 | CH ₃ | 22.5 | CH ₃ | 32.6 | CH ₃ | 111.5 | CH ₂ |
| 15 | 24.9 | CH ₃ | 24.0 | CH ₃ | 26.4 | CH ₃ | 14.0 | CH ₃ |
| 1' | | | 55.4 | CH ₂ | | | | |
| 2' | | | 16.2 | CH ₃ | | | | |

Compounds 1, 3, and 4 were measured in $\text{C}_5\text{D}_5\text{N}$, and 2 was measured in CDCl_3 .

Compound 2 was obtained as a colorless syrup. The molecular formula of 2 was $\text{C}_{17}\text{H}_{28}\text{O}_4$ as determined by the HRESIMS peak at m/z 615.3872 ($[2\text{M} + \text{Na}]^+$, calcd. for 615.3873) with four degrees of unsaturation. The ^1H NMR data (Table 1) show signals of four tertiary methyls at δ_{H} 1.22 (d, H₃-13), 1.15 (s, H₃-14), 1.33 (s, H₃-15), and 1.11 (t, H₃-2') and an oxygenated methine at δ_{H} 4.23 (H-6) and an oxygenated methylene at 3.37 (H₂-1'). The ^{13}C NMR data of 2 (Table 2) identify 17 resonances ascribed to four methyls, five methylenes (one oxygenated), five methines (one oxygenated), two oxygenated tertiary carbons, and one ester carbonyl carbon. The ^1H and ^{13}C NMR data of 2 (Tables 1 and 2) display characteristic signals of a guaiane-type sesquiterpenoid, which quite resembled that of α -Hydroxy-10 α -methoxyguaian-12,6-olide [13]. A careful comparison of ^1H and ^{13}C NMR data and molecular formula showed that ethoxyl, rather than methoxyl, existed at C-10 in 2. The relative configuration of 2 was deduced from the NOE correlations (Figure 3). The cross peaks of H-1/H-5, H-5/H-7, and H-7/H₃-13 indicated that these protons were cofacial and α -oriented. The cross peaks of H-6/H₃-16, H-6/H₃-14, and H-6/H-11 proved that they were on the opposite face and were assigned to be β -oriented. Accordingly, compound 2 was determined structurally as 4 α -Hydroxy-10 α -ethoxyguaian-12,6-olide.

**Figure 3.** Key ^1H - ^1H COSY, HMBC, and NOESY correlations of 2.

Compound 3 was obtained as a colorless crystal plate. The molecular formula of 3 was $\text{C}_{15}\text{H}_{26}\text{O}_3$ as determined by the HRESIMS peak at m/z 253.1803 ($[\text{M} - \text{H}]^-$, calcd. for 253.1804). The ^1H NMR data (Table 1) show signals of three tertiary methyls at δ_{H} 1.42 (H₃-13), 1.43 (H₃-14), and 1.73 (H₃-15). The ^{13}C NMR data of 3 (Table 2) identify 15 resonances ascribed to three terminal methyls, five methylenes (one oxygenated), four methines, and three tertiary carbon (two oxygenated). Taking into account the molecular formula, a tricyclic skeleton was indicated. The presence of one characteristic quaternary carbon at

high field (C-11, δ_C 24.9) displayed it is an aromadendrane-type sesquiterpene [14,15]. The HMBC correlations from H₂-12 (δ_H 4.06 and 4.15) to C-11, C-13, C-6, and C-7 and from H₃-13 (δ_H 1.42) to C-11, C-12, C-6, and C-7 verified the existence of a cyclopropyl unit at C-6 and C-7. Moreover, The HMBC correlations from H₃-14 (δ_H 1.43) to C-1, C-9, and C-10 and from H₃-15 (δ_H 1.73) to C-3, C-4, and C-5 established the planar structure of compound 165 as aromadendrane-4,10,12-triol. The relative configurations of the six chiral centers at C-1, C-4, C-5, C-6, C-7, and C-9 in **3** were similar to those in (–)-alloaromadendrane-4 β ,10 β -diol [16], which was confirmed by the ROESY correlations of H-1 with H-5 and H₂-12; H-6 with H₃-11; and H-7 with H-8 in its ROESY spectrum (Figure 4). Therefore, compound **3** was characterized as (–)-alloaromadendrane-4 β ,10 β ,12-triol.

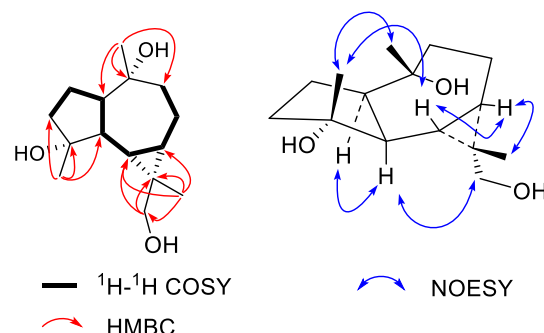


Figure 4. Key ^1H - ^1H COSY, HMBC, and NOESY correlations of **3**.

Compound **4** was obtained as a colorless viscous oil. The molecular formula of **4** was $\text{C}_{15}\text{H}_{22}\text{O}_4$ as determined by the HRESIMS peak at m/z 265.1441 ($[\text{M} - \text{H}]^+$, calcd. for 265.1440). In ^1H NMR, it can be recognized that there are two methyl groups at δ_H 1.11 and 0.75 (each d, 3H) and an exocyclic methylene at δ_H 5.64 and 4.93. The ^{13}C NMR spectrum indicated 15 carbon resonances (Table 2), which contained two carbonyl carbons, two downfield alkene carbons, an oxygenated carbon, two methyls, two methylenes, and five methines. A long coupling carbon chain from C-3 to C-9 that extended from C-8 to C-13 was deduced by the ^1H - ^1H COSY correlations (Figure 5). The HMBC correlations from H₃-13 to C-8, C-11, and C-12 and from H-9 to C-12, as well as from H₃-14 to C-1, C-9, and C-10, established a preguaiene-type sesquiterpene containing α -methyl- γ -lactone. The relative configuration of **4** was concluded from the NOESY experiment (Figure 5). The cross peaks of H-1/H₃-15, H-7 α /H₃-15, and H-7 α /H₃-13 indicated that they were cofacial and α -oriented. The cross peaks of H-8/H-9 and H-8/H-11 sustained that these protons were on the other face and assigned to be β -oriented. Therefore, compound **4** was characterized as depicted and named preambrosia A.

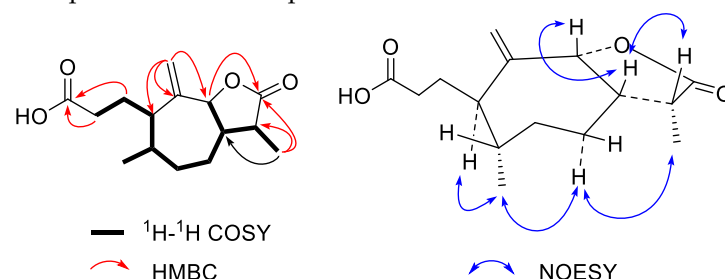


Figure 5. Key ^1H - ^1H COSY, HMBC, and NOESY correlations of **4**.

Known compounds were identified by comparison with the experimental and reported spectroscopic data as follows: (–)-compressanolide (**5**) [17], magnograndiolide (**6**) [18], 1 β ,7 β ,9 β ,10 β ,13 α H-guaia-4(5)-en-12,6 β -olide-9- O - β -D-glucoside (**7**) [19], tetrahydrocoropilin (**8**) [8], psilostachyin C (**9**) [20], psilostachyin B (**10**) [21], 11 α ,13-dihydroperuvine (**11**) [22], 3 α ,4 β -dihydrocumanin (**12**) [23], dihydrocumanin diacetate (**13**) [23], dihydrocumanin acetonide (**14**) [23], damsinic acid (**15**) [24], ambrosic acid (**16**) [25], 11,13-dihydroparthenolide

(17) [26], 11 β ,13-dihydro-14-hydroxyparthenolide (18) [26], (-)-9 α -hydroxydihydroparthenolid (19) [26], and (+)-alloaromadendrane-4 β ,10 α -diol (20) [27]. The structure of these isolated sesquiterpenoids can be divided into guaiane-, pseudoguaiane-, secopseudoguaiane-, germacrene-, and aromadendrane-type carbon skeletons. Among them, guaiane- and pseudoguaiane-type sesquiterpenoids are the most characteristic and diverse subclass in the inflorescence of *A. artemisiifolia*. Aromadendrane-type sesquiterpenes are rare in the genus *Ambrosia* [28]; however, aromadendranols 3 and 20 were isolated from the inflorescence of *A. artemisiifolia*.

2.2. Allelopathy Activity

Compounds 1–20 were evaluated for their allelopathic activities on the seedling growth of wheat (*T. Aestivum*) at a concentration of 200 $\mu\text{g}/\text{mL}$. Our results show that fourteen compounds (1–4, 5, 9–12, 14–15, 17, and 19–20) inhibited the root growth of the tested seedlings to varying degrees. Compounds 14, 15, 17, and 20 significantly inhibited root length, which was more than 50% shorter than that of the control (Figure 6). In contrast, the inhibitory effect of these active compounds on shoot growth was reasonably weak, with relative shoot length being no less than 60% of the control (Figure 7). It is notable that compound 14, a pseudoguaiane-type sesquiterpene, showed the most potent allelopathic activity both on root and shoot length. Comparing the structure–activity relationships of compounds 11–14 showed that 14 had an acetonide group on the adjacent hydroxyl of C-3/C-4, which may be related to a high allelopathic effect. Previous research has demonstrated that parthenolides, germacrene-type sesquiterpenes, have strong allelopathic inhibitory activity [29]. The structural analogue of 17 also showed good inhibitory activity, but compounds 18 and 19 with an additional hydroxyl at C-14 and C-9, respectively, exhibited very weak allelopathic activities.

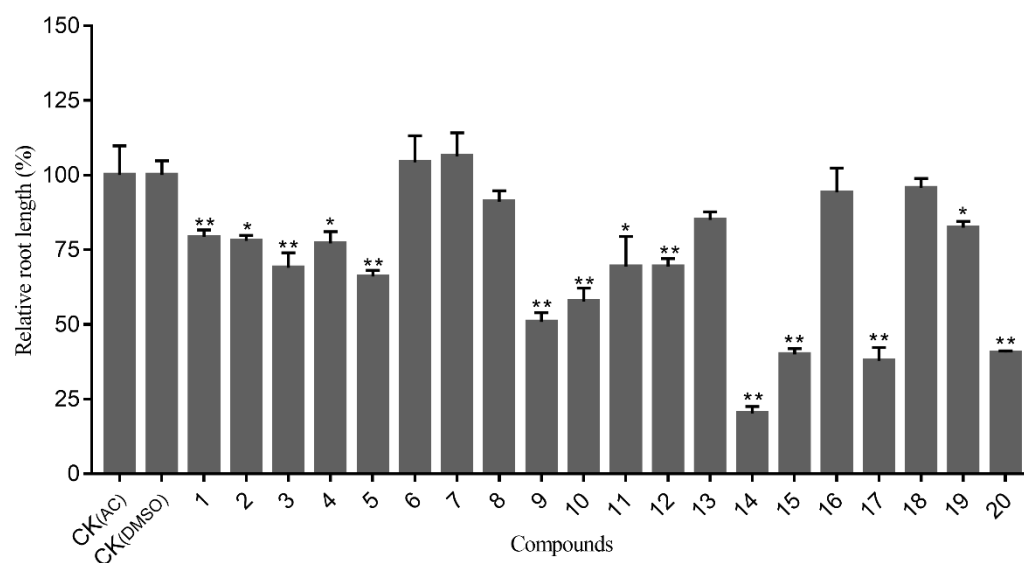


Figure 6. Allelopathic activity of sesquiterpenoids (1–20) on root length of wheat. Note: CK_(AC) = acetone solvent control and CK_(DMSO) = DMSO solvent control; the compounds were solubilized with acetone, except 1, 7, and 9 were solubilized with DMSO. Each compound's result was compared with the solvent control via independent sample *t*-test; * indicates significant difference ($P < 0.05$); ** indicates extremely significant difference ($P < 0.01$).

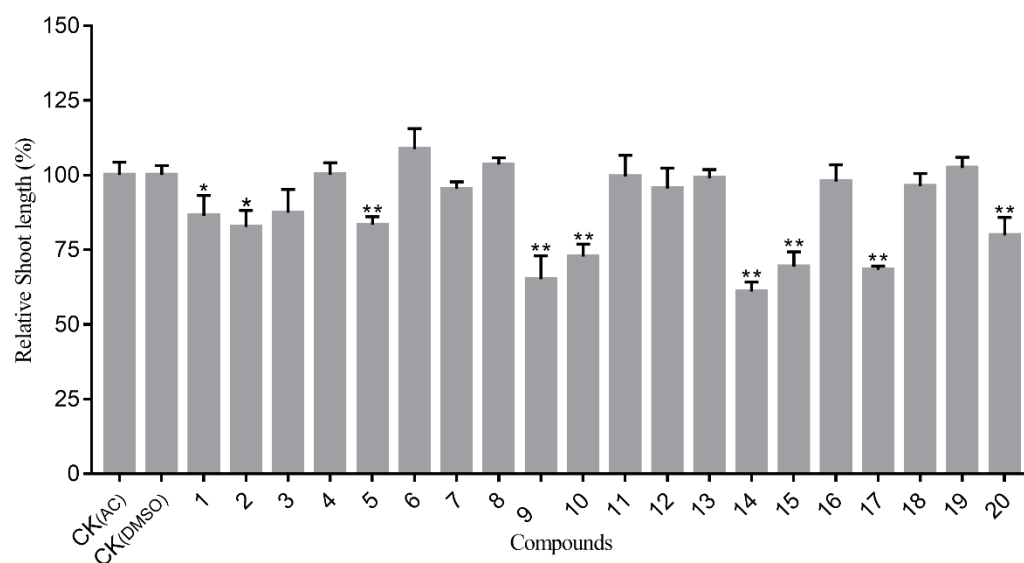


Figure 7. Allelopathic activity of sesquiterpenoids (1–20) on shoot length of wheat. Note: CK (AC) = acetone solvent control and CK (DMSO) = DMSO solvent control; the compounds were solubilized with acetone, except 1, 7, and 9 were solubilized with DMSO. Each compound's result was compared with the solvent control via independent sample *t*-test; * indicates significant difference ($P < 0.05$); ** indicates extremely significant difference ($P < 0.01$).

Sesquiterpenoids, which play an important role in plant allelopathy, are abundant in many invasive plants and can significantly inhibit the growth of neighboring plants [9,10]. On the other hand, allelopathic components generally have strong phytotoxic activities against many weeds [30,31]. Our findings reveal the chemical profile of the sesquiterpenoids occurring in the inflorescence of *A. artemisiifolia*, and our bioassay results are useful in understanding the chemical basis of allelopathy in *A. artemisiifolia*.

3. Materials and Methods

3.1. General

Melting points were obtained on a SGW X-4 micromelting point apparatus (INESA Co., Shanghai, China). Optical rotations were measured on SGW-533 automatic polarimeter (INESA Co., Shanghai, China). HRESIMS spectra were taken on an API QSTAR mass spectrometer (Applied Biosystem/MSD Sciex, Concord, ON, Canada). The 1D- and 2D-NMR spectra were recorded on a Bruker Avance III 600MHz NMR spectrometer using TMS as an internal standard. Column chromatography was performed on silica gel (100–200 mesh, Qingdao Marine Chemical Ltd., Qingdao, China). Preparative TLC plates (HSGF254, Jiangyou silicone Development Co., Ltd., Yantai, China), Sephadex LH-20 (GE Healthcare, Uppsala, Sweden), and Develosil ODS (50 μ m, Nomura Chemical Co. Ltd., Osaka, Japan) were used for the isolation experiments. Preparative HPLC was performed on a Waters 1525 Binary HPLC pump and a Waters 2414 refractive index detector (Waters Corp, Milford, MA, USA) using a YMC-Pack ODS-A column (250 mm \times 10 mm I.D.; S-5 μ m, 12 nm).

3.2. Plant Material

The inflorescence of *A. artemisiifolia* was collected from Cangwu County, Guangxi province, China, in August 2019, which was identified by Prof. Dai-gui Zhang (Key laboratory of Plant Resources Conservation and Utilization, Jishou University). A voucher specimen (zdg20190801) was deposited in Hunan Agricultural University. Seeds of *Triticum aestivum* L. were purchased from a seed company (Jiangsu Dingsheng Seed Co., Zhenjiang, China).

3.3. Extraction and Isolation

The air-dried and powdered inflorescence of *A. artemisiifolia* (9.0 kg) was extracted with petroleum ether (10 L × 4) and then extracted with ethanol (95%, 10 L × 4). The ethanol extract was suspended in water and partitioned with EtOAc and *n*-butanol successively, as described in our previous research [32].

The petroleum ether extract (254.9 g) was subjected to silica gel column chromatography (CC, 100–200 mesh, 2000 g) eluted successively with gradient petroleum ether–acetone mixtures of increasing polarity (100:0 → 60:40, v/v) to separate fifteen fractions (PE.A1–PE.A15). A large number of crystals precipitated in PE.A8, and compound **17** (1100.4 mg) was obtained by recrystallization.

The EtOAc extract (370.0 g) was separated into twelve fractions (ET.A1–ET.A12) by a silica gel column (100–200 mesh, 1000 g) eluted with an increasing gradient of CHCl₃–MeOH mixtures.

Fraction ET.A2 (165.7 g) was decolorized on an MCI gel column to obtain a yellowish syrup subfraction A2B (133.0 g) from the 90% MeOH eluent. A2B was then subjected to silica gel CC (200–300 mesh, 1000 g) eluted carefully with a gradient of petroleum ether–acetone mixtures, yielding nine fractions Fr. B1–B9. These fractions (B1–B9) were first chromatographed on an ODS-C18 column eluting with MeOH–H₂O (30:70 → 90:10, v/v) to produce seven subfractions. Each of the subfractions was further purified by a sephadex LH-20 column eluting with MeOH to collect the points with color features of terpenoids on TLC. Finally, three major points were obtained from subfraction B2 (10.3 g), of which compound **15** (157.4 mg) was directly crystallized after sephadex LH-20 CC, and compounds **5** (15.3 mg, Rt = 17 min) and **3** (103.2 mg, Rt = 28 min) were yielded by semipreparative HPLC (55% MeOH /H₂O, 3 mL/min). Two major points were obtained from subfraction B3 (12.8 g), yielding compounds **13** (73.1 mg, R_f = 0.3) and **2** (12.6 mg, R_f = 0.5) by TLC preparation with CHCl₃–MeOH (90:10) as the developing solvent. Four major points were obtained from subfraction B4 (20.4 g), and compounds **16** (571.8 mg) and **9** (43.6 mg) were crystallized after sephadex LH-20 CC; compounds **8** (22.5 mg, R_f = 0.4) and **11** (10.2 mg, R_f = 0.6) were yielded by TLC preparation using CHCl₃–MeOH (95:5) as the developing solvent. Two major points were obtained from subfraction B5 (12.8 g), which obtained compounds **4** (24.8 mg, R_f = 0.2) and **20** (12.2 mg, R_f = 0.5) by TLC preparation with petroleum ether–acetone (90:10) as the developing solvent. Three major points were obtained from subfraction B6 (11.8 g), and compound **12** (238 mg) was crystallized after sephadex LH-20 CC; compounds **1** (15.8 mg, Rt = 36 min) and **14** (20.0 mg, Rt = 45 min) were yielded by semipreparative HPLC (45% MeOH /H₂O, 3 mL/min). Three major points were obtained from subfraction B7 (10.5 g), and compounds **6** (13.0 mg, Rt = 18 min), **18** (15.1 mg, Rt = 22 min), and **19** (28.2 mg, Rt = 30 min) were yielded by semipreparative HPLC (30% MeOH/H₂O, 3 mL/min).

Similarly, fractions ET.A9 (6.3 g) and ET.A10 (6.8 g) were chromatographed on ODS-C18 followed by Sephadex LH-20 (MeOH) to collect clear spots using TLC detection. Compound **10** (5.8 mg) was obtained from ET.A9 by TLC preparation with ether–acetone (90:15) as the developing solvent, while compound **7** (15.3 mg) was obtained from ET.A10 by TLC preparation using ether–acetone (80:20) as the developing solvent.

3.4. Physical and Chemical Data of 1–4

Compound **1**: yellowish syrup, $[\alpha]_D^{25} - 62.0$ (c 0.6, MeOH); ¹H NMR (600 MHz, C₅D₅N) and ¹³C NMR (150 MHz, C₅D₅N) spectroscopic data, see Tables 1 and 2; positive ion ESIMS *m/z*: 291 [M + Na]⁺; negative ESIMS *m/z*: 267 [M – H][–]; HRESIMS *m/z*: 267.1598 [M – H][–] (calcd. for C₁₅H₂₃O₄, 267.1596). (Data from Figures S1–S7).

Compound **2**: colorless syrup, $[\alpha]_D^{25} - 43.0$ (c 0.8, MeOH); ¹H NMR (600 MHz, CDCl₃) and ¹³C NMR (150 MHz, CDCl₃) spectroscopic data, see Tables 1 and 2; positive ion ESIMS *m/z*: 319 [M + Na]⁺, 615 [2M + Na]⁺; HRESIMS *m/z*: 615.3872 [2M + Na]⁺ (calcd. for C₃₄H₅₆O₈Na, 615.3873). (Data from Figures S8–S14).

Compound **3**: colorless crystal plate, mp 305–307 °C, $[\alpha]_D^{25} + 14.0$ (c 0.1, MeOH); ¹H NMR (600 MHz, C₅D₅N) and ¹³C NMR (150 MHz, C₅D₅N) spectroscopic data, see

Tables 1 and 2; negative ESIMS m/z : 253 $[M - H]^-$, 289 $[M + Cl]^-$; HRESIMS m/z : 253.1803 ($[M - H]^-$ (calcd. for $C_{15}H_{25}O_3$, 253.1804). (Data from Figures S15–S21).

Compound 4: colorless viscous oil, $[\alpha]_D^{25} - 26.0$ (c 0.6, MeOH); 1H NMR (600 MHz, C_5D_5N) and ^{13}C NMR (150 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; positive ion ESIMS m/z : 267 $[M + H]^+$, 289 $[M + Na]^+$; negative ESIMS m/z : 265 $[M - H]^-$, 301 $[M + Cl]^-$; HRESIMS m/z : 265.1441 ($[M - H]^-$ (calcd. for $C_{15}H_{21}O_4$, 265.1440). (Data from Figures S22–S28).

3.5. Bioassays

Inhibitory activities on the growth of *T. aestivum* seedlings were evaluated using the plate culture method [33]. To enhance the germination rate, *T. aestivum* seeds were submerged in a 0.3% potassium permanganate solution for 15 min before being thoroughly rinsed with distilled water. After skin breaking, *T. aestivum* seeds were germinated on filter paper in the dark at 25 °C for 24 h. After being dissolved in acetone or DMSO, each compound (1–20) was prepared as a stock solution of 2 mg/mL. The stock solutions were diluted with distilled water (containing 1% Tween 80) to obtain concentrations of 200 µg/mL. The same volume of acetone or DMSO was added to distilled water (containing 1% Tween 80) as a control. Following germination, uniformly growing seedlings (10 seeds) were transferred to 9 cm diameter Petri dish lined with filter paper. Each dish was treated with 7 mL of the prepared corresponding concentration of test solution (or control solution). Each treatment was replicated 3 times. The seedlings were then incubated in a constant temperature humidity chamber in the dark at 25 °C for 72 h. At the end of the experiment, root and stem lengths were measured.

3.6. Statistical Analysis

All data were subjected to analysis of variance by use of SPSS 18.0. Significant differences in seedling growth between treatment and control were calculated by one-way analysis of variance (ANOVA). Relative length (percent) was determined by the formula $[\text{treated length}/\text{control length}] \times 100$.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules27185915/s1>, Figures S1–S7: HR-ESIMS, 1D and 2D-NMR spectra of compound 1; Figures S8–S14: HR-ESIMS, 1D and 2D-NMR spectra of compound 2; Figures S15–S21: HR-ESIMS, 1D and 2D-NMR spectra of compound 3; Figures S22–S28: HR-ESIMS, 1D and 2D-NMR spectra of compound 4.

Author Contributions: Conceptualization, Y.L. and W.D.; methodology, Z.Z.; software, H.H. (Hualiang He); validation, L.Q. and Q.G.; formal analysis, H.H. (Hong Huang); investigation, Z.Z. and H.H. (Hong Huang); resources, H.H. (Hong Huang); data curation, W.D.; writing—original draft preparation, Z.Z.; writing—review and editing, W.D.; visualization, Y.L.; supervision, W.D.; project administration, W.D.; funding acquisition, W.D. and Y.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Key Research and Development Program of China (Grant No. 2021YFD1401100); the Key Research and Development Program of Hunan Province (China) (Grant No. 2020NK2034); and the Hunan Provincial Natural Science Foundation of China (Grant No. 2021JJ30317).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data included in this study are available upon request by contact with the corresponding author.

Acknowledgments: We thank Dai-gui Zhang (Key laboratory of Plant Resources Conservation and Utilization, Jishou University, China) for his assistance in the identification of plant materials.

Conflicts of Interest: The authors state no conflict of interest.

Sample Availability: Samples of the compounds are not available from the authors.

References

1. Montagnani, C.; Gentili, R.; Smith, M.; Guarino, M.F.; Citterio, S. The worldwide spread, success, and impact of ragweed (*Ambrosia* spp.). *Crit. Rev. Plant Sci.* **2017**, *36*, 139–178. [[CrossRef](#)]
2. Sun, Y.; Roderick, G.K. Rapid evolution of invasive traits facilitates the invasion of common ragweed, *Ambrosia artemisiifolia*. *J. Ecol.* **2019**, *107*, 2673–2687. [[CrossRef](#)]
3. Putnam, A.R.; Tang, C.S. *The Science of Allelopathy*; Wiley: New York, NY, USA, 1986; pp. 1–9.
4. Macías, F.A.; Mejías, F.J.R.; Molinillo, J.M.G. Recent advances in allelopathy for weed control: From knowledge to applications. *Pest Manag. Sci.* **2019**, *75*, 2413–2436. [[CrossRef](#)] [[PubMed](#)]
5. Bonea, D.; Bonciu, E.; Niculescu, M.; Olaru, A.L. The allelopathic, cytotoxic and genotoxic effect of *Ambrosia artemisiifolia* on the germination and root meristems of *Zea mays*. *Caryologia* **2018**, *71*, 24–28. [[CrossRef](#)]
6. Vidotto, F.; Tesio, F.; Ferrero, A. Allelopathic effects of *Ambrosia artemisiifolia* L. in the invasive process. *Crop Prot.* **2013**, *54*, 161–167. [[CrossRef](#)]
7. An, J.P.; Ha, T.K.Q.; Kim, H.W.; Ryu, B.; Kim, J.; Park, J.; Lee, C.H.; Oh, W.K. Eudesmane glycosides from *Ambrosia artemisiifolia* (common ragweed) as potential neuroprotective agents. *J. Nat. Prod.* **2019**, *82*, 1128–1138. [[CrossRef](#)]
8. Tagliatalata-Scafati, O.; Pollastro, F.; Minassi, A.; Chianese, G.; De Petrocellis, L.; Di Marzo, V.; Appendino, G. Sesquiterpenoids from common ragweed (*Ambrosia artemisiifolia* L.), an invasive biological pollutant. *Eur. J. Org. Chem.* **2012**, *27*, 5162–5170. [[CrossRef](#)]
9. Bennett, R.N.; Wallsgrove, R.M. Secondary metabolites in plant defence mechanisms. *New Phytol.* **1994**, *127*, 617–633. [[CrossRef](#)]
10. Chadwick, M.; Trewin, H.; Gawthrop, F.; Wagstaff, C. Sesquiterpenoids lactones: Benefits to plants and people. *Int. J. Mol. Sci.* **2013**, *14*, 12780–12805. [[CrossRef](#)]
11. Zhao, F.; Durner, J.; Winkler, J.B.; Traidl-Hoffmann, C.; Strom, T.M.; Ernst, D.; Frank, U. Pollen of common ragweed (*Ambrosia artemisiifolia* L.): Illumina-based de novo sequencing and differential transcript expression upon elevated NO₂/O₃. *Environ. Pollut.* **2017**, *224*, 503–514. [[CrossRef](#)]
12. Yu, S.-J.; Yu, J.-H.; Yu, Z.-P.; Yan, X.; Zhang, J.-S.; Sun, J.-Y.; Zhang, H. Bioactive terpenoid constituents from *Eclipta prostrata*. *Phytochemistry* **2020**, *170*, 112192. [[CrossRef](#)] [[PubMed](#)]
13. Fischer, N.H.; Lu, T.; Cantrell, C.L.; Castañeda-Acosta, J.; Franzblau, S.G. Antimycobacterial evaluation of germacranolides in honour of professor G.H. Neil Towers 75th birthday. *Phytochemistry* **1998**, *49*, 559–564. [[CrossRef](#)]
14. Cabrera, G.M.; Oberti, J.C.; Gros, E.G.; Seldes, A.M. Aromadendrane sesquiterpene from *Mandevilla pentlandiana*. *Phytochemistry* **1993**, *34*, 1642–1643. [[CrossRef](#)]
15. Gijzen, H.J.M.; Wijnberg, J.B.P.A.; De Groot, A. Structure, occurrence, biosynthesis, biological activity, Synthesis, and chemistry of aromadendrane sesquiterpenoids. *Fortschr. Chem. Org. Naturst.* **1995**, *64*, 149–193. [[CrossRef](#)]
16. Sun, Z.-H.; Hu, C.-Q.; Wang, J.-Y. A new sesquiterpene from *Caragana intermedia*. *Chin. Chem. Lett.* **2007**, *18*, 1379–1382. [[CrossRef](#)]
17. Bargues, V.; Blay, G.; Cardona, L.; García, B.; Pedro, J.R. Stereoselective synthesis of (+)-11βH,13-dihydroestafiatin, (+)-11βH,13-dihydrodudartin, (–)-compressanolide, and (–)-11βH,13-dihydromicheliolide from santonin. *J. Nat. Prod.* **2002**, *65*, 1703–1706. [[CrossRef](#)]
18. Jacobsson, U.; Kumar, V.; Saminathan, S. Sesquiterpene lactones from *Michelia champaca*. *Phytochemistry* **1995**, *39*, 839–843. [[CrossRef](#)]
19. Ding, W.-B.; Huang, R.; Zhou, Z.-S.; Li, Y.-Z. New sesquiterpenoids from *Ambrosia artemisiifolia* L. *Molecules* **2015**, *20*, 4450–4459. [[CrossRef](#)]
20. Sülsen, V.P.; Frank, F.M.; Cazorla, S.I.; Barrera, P.; Freixa, B.; Vila, R.; Sosa, M.A.; Malchiodi, E.L.; Muschietti, L.V.; Martino, V.S. Psilostachyin C: A natural compound with trypanocidal activity. *Int. J. Antimicrob. Agents* **2011**, *37*, 536–543. [[CrossRef](#)]
21. Mabry, T.J.; Kagan, H.B.; Miller, H.E. Psilostachyin B, a new sesquiterpene dilactone from *Ambrosia psilostachya* DC. *Tetrahedron* **1996**, *22*, 1943–1948. [[CrossRef](#)]
22. Fischer, N.H.; Weidenhamer, J.D.; Riopel, J.L.; Quijano, L.; Menelaou, M.A. Stimulation of witchweed germination by sesquiterpene lactones: A structure-activity study. *Phytochemistry* **1990**, *29*, 2479–2483. [[CrossRef](#)]
23. Romo, J.; Joseph-Nathan, P.; Siade, G. The structure of cumanin, a constituent of *Ambrosia cumanensis*. *Tetrahedron* **1966**, *22*, 1499–1506. [[CrossRef](#)]
24. Ohmoto, T.; Ikeda, K.; Nomura, S.; Shimizu, M.; Saito, S. Studies on the sesquiterpenes from *Ambrosia elatior* Linne. *Chem. Pharm. Bull.* **1987**, *35*, 2272–2279. [[CrossRef](#)]
25. Oberti, J.C.; Silva, G.L.; Sosa, V.E.; Kulanthaivel, P.; Herz, W. Ambrosanolides and secoambrosanolides from *Ambrosia tenuifolia*. *Phytochemistry* **1986**, *25*, 1355–1358. [[CrossRef](#)]
26. Galal, A.M.; Ibrahim, A.-R.S.; Mossa, J.S.; El-Ferali, F.S. Microbial transformation of parthenolide. *Phytochemistry* **1999**, *51*, 761–765. [[CrossRef](#)]
27. Goldsby, G.; Burke, B.A. Sesquiterpene lactones and a sesquiterpene diol from jamaican *Ambrosia peruviana*. *Phytochemistry* **1987**, *26*, 1059–1063. [[CrossRef](#)]
28. Kovács, B.; Hohmann, J.; Csupor-Löffler, C.; Kiss, T.; Csupor, D. A comprehensive phytochemical and pharmacological review on sesquiterpenes from the genus *Ambrosia*. *Heliyon* **2022**, *8*, e09884. [[CrossRef](#)]

29. Macías, F.A.; Galindo, J.C.G.; Massanet, G.M. Potential allelopathic activity of several sesquiterpene lactone models. *Phytochemistry* **1992**, *31*, 1969–1977. [[CrossRef](#)]
30. Anese, S.; Jatobá, L.J.; Grisi, P.U.; Gualtieri, S.C.J.; Santos, M.F.C.; Berlinck, R.G.S. Bioherbicidal activity of drimane sesquiterpenes from *Drimys brasiliensis* Miers roots. *Ind. Crops Prod.* **2015**, *74*, 28–35. [[CrossRef](#)]
31. Masi, M.; Pannacci, E.; Santoro, E.; Zermane, N.; Superchi, S.; Evidente, A. Stoechanones A and B, phytotoxic copaane sesquiterpenoids isolated from *Lavandula stoechas* with potential herbicidal activity against *Amaranthus retroflexus*. *J. Nat. Prod.* **2020**, *83*, 1658–1665. [[CrossRef](#)]
32. Zeng, Z.; Cheng, D.; Lai, M.M.; He, H.L.; Qiu, L.; Xue, J.; Li, Y.Z.; Ding, W.B. A new lignan and C (6)-oxygenated flavonoids from the inflorescence of *Ambrosia artemisiifoli*. *Chem. Biodiversity* **2022**, *19*, e202100897. [[CrossRef](#)] [[PubMed](#)]
33. Guo, K.; He, X.-F.; Yan, Z.-Q.; Li, X.-Z.; Ren, X.; Pan, L.; Qin, B. Allelochemicals from the rhizosphere soil of cultivated *Astragalus hoantchy*. *J. Agric. Food Chem.* **2016**, *64*, 3345–3352. [[CrossRef](#)] [[PubMed](#)]