Secondary Metabolites and Their Cytotoxic Activity of Artemisia nitrosa Weber. and Artemisia marschalliana Spreng.

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Abstract: As a promising source of biologically active substances, the Artemisia species from Kazakhstan have not been investigated efficiently. Considering the rich history, medicinal values, and availability of the Artemisia plants, systematic investigations of two Artemisia species growing in the East Kazakhstan region were conducted. In this study, one new germacrane-type sesquiterpene lactone (11), together with 10 known sesquiterpenes and its dimer, were characterized from A. nitrosa Weber. Additionally, one new chromene derivative (1’) with another 12 known compounds, including coumarins, sesquiterpene diketones, phenyl propanoids, polyacetylenics, dihydroxycinnamic acid derivatives, fatty acids, naphthalene derivatives, flavones, and caffeic acid derivatives were isolated from A. marschalliana Spreng. All compounds were isolated and identified for the first time from these two Artemisia species. The structures of new compounds (11, 1’) were established by using UV, TOFMS, LC–MS, 1D and 2D NMR spectroscopic analyses. The cytotoxicity of all isolated compounds was evaluated. As a result, all compounds did not show significant inhibition against HL-60 and A-549 cell lines. The sesquiterpenoids isolated from A. nitrosa were tested for their inhibitory activity against the LPS-induced NO release from the RAW624.7 cells, and neither of them exhibited significant activity.

Keywords: Artemisia nitrosa; Artemisia marschalliana; sesquiterpene lactone; coumarin; flavonoid; phenolic compound; cytotoxicity; HL-60; A-549

1. Introduction

The Artemisia species are perennial high-vascular plants and have been used for centuries in traditional medicine [1]. The medicinal benefits of the Artemisia genus include normalizing the work of the gastrointestinal tract, especially in gastritis with low acidity, increasing appetite [2–4], and treating bronchial asthma [5], rheumatism [6], dermatitis [7,8], malaria [9], etc. Moreover, a few scientific publications reported that some natural Artemisia drugs showed promising potential to cure diseases, such as AIDS, cancer, cardiovascular diseases, and renal disorders [10–12]. Extensive research has resulted in the isolation of a number of bioactive secondary metabolites, such as essential oils, flavonoids, terpenes, esters, and phenolic [13,14]. Many compounds from the genus showed antimalarial, antiviral, anticancer, antipyretic, antimembranous, anticoagulant, antiangiinal, antioxidant, antilucre, and antisapmodic properties [1,15–17].

Artemisia is one of the largest genera in the Asteraceae family, encompassing more than 400 species, and is widely distributed all over the world [18,19]. The most significant number of species are found in Russia and China and, in Kazakhstan, 81 species were documented, with 19 being endemic, and 34 growing in the territory of Central
Kazakhstan [20,21]. As is well known, plants of the Artemisia species have a history of manufacturing potentially cytotoxic substances. For instance, the Central Asian oncology clinics use a sesquiterpene lactone named arglabin, derived from the A. glabella plant growing in Central Kazakhstan, to treat various cancers [22]. The pharmacologically active flavone eupatrilin, which was isolated from A. asiatica, has cytotoxic and chemopreventive properties [23]. Our previous work on the endemic A. heptaptamica in the Almaty region of Kazakhstan revealed 13 sesquiterpene lactones, most of which showed potent inhibition against the activation of NF-κB induced by LPS [24].

Another recent study of our group has revealed that methanolic extracts of a total of nine Artemisia species from Central Asia showed a high potential for α-glucosidase, PTP1B, antioxidant, and BNA inhibition, which are associated with diabetes, obesity, and bacterial infections. Of these, both A. nitrosa and A. marschalliana exhibited a PTP1B inhibition around 75% at a concentration of 50 µg/mL. Similarly, both Artemisia species also showed the highest activities (>85%) against BNA even at lower concentration of 20 µg/mL [21].

Artemisia nitrosa Weber is native to saline desert-steppe landscapes of Kazakhstan, southern Siberia, and Mongolia, with secondary distribution in Transbaikalia [25]. However, A. nitrosa is a poorly studied plant. Secondary metabolites of A. nitrosa, such as sesquiterpene lactones and dimers, are being isolated and identified for the first time by our research team.

Artemisia marshalliana Spreng is found in steppe meadows, steppes, and pine forests throughout the Far East, Siberia, the Caucasus, and Kazakhstan [20]. It is an Iranian traditional medicinal plant whose extracts showed antibacterial and anticancer properties in human gastric carcinoma (AGS) and L929 cell lines, while the essential oil has antimalarial properties [26,27].

This study aimed to phytochemically investigate non-explored Artemisia species in Kazakhstan, and resulted in the characterization of germacrene-type sesquiterpene lactones from A. nitrosa and phenolic compounds from A. marschalliana, including a total of 2 new and 23 known compounds for the first time. Their structures have been established using extensive analyses of UV, MS, 1D, and 2D NMR spectroscopic data. All compounds were evaluated for cytotoxicity against human cancer cell lines HL-60 and A-549.

2. Materials and Methods
2.1. General Experimental Procedures

To distinguish a certain substance, the combination of NMR (1D and 2D) analytical techniques with other experimental methods, such as LC–MS, UV, IR, preparative HPLC, and semi-preparative HPLC were used. A Shimadzu UV-2550 UV–vis spectrophotometer is used for the measurement of UV spectra. The IR spectra are registered on a Thermo Nicolet FTIR IS 5 spectrophotometer. The HR-ESIMS spectra were measured on a Waters Synapt G2-Si Q-TOF instrument with a Waters BEH C18 column (1.7 µm, 2.1 mm × 50 mm, CH3CN:H2O with 0.1% formic acid, from 5% to 95%, 0–9 min, flow rate 0.4 mL/min, 45 °C). Analytical HPLC was performed on a Waters e2695 system equipped with a Waters 2998 photodiode array detector (PDA), a Waters 2424 evaporative light-scattering detector (ELSD), and a Waters 3100 MS detector, using a Waters Sunfire RP C18 column (5 µm, 4.6 mm × 150 mm, CH3CN:H2O with 0.1% formic acid, from 5% to 95%, 0–25 min, flow rate 1.0 mL/min, 30 °C). Preparative HPLC was run on a Waters system equipped with a Waters 2767 autosampler, a Waters 2545 pump, a Waters 2489 PDA and an Acuity ELSD using a Waters Sunfire RP C18 column (5 µm, 30 mm × 150 mm, flow rate 30 mL/min).

The NMR spectra were recorded on a Bruker Avance III (Bruker, Zurich, Switzerland) using a 500 M NMR spectrometer with TMS as the internal standard. The chemical shift (δ) values were given in ppm and coupling constants (J) in Hz. All solvents used for CC were of at least analytical grade (Shanghai Chemical Reagents Co., Ltd., Shanghai, China), and solvents used for HPLC were of HPLC grade (Merck KGaA, Darmstadt, Germany).

Column chromatography (CC) was performed on MCI gel CHP20P (75–150 µm, Mitsubishi Chemical Industries, Tokyo, Japan), Econosep C18 60A (50 µm, DIKMA, Beijing,
China), Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), and silica gel (100–200 and 300–400 mesh, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China). The TLC was carried out on precoated silica gel 60 F254 aluminum sheets (Merck, Darmstadt, Germany), and the TLC spots were viewed at 254 nm and visualized using 5% sulfuric acid in alcohol containing 10 mg/mL of vanillin.

2.2. Plant Materials

Here, *A. nitrosa* and *A. marschalliana* were gathered from East Kazakhstan at the end of July 2020 and identified by experts of the Republican State Enterprise on the subject of economic management at the “Institute of Botany and Phytointroduction” of the Committee of Forestry and Wildlife of the Ministry of Ecology, Geology, and Natural Resources of the Republic of Kazakhstan. A sample of *A. nitrosa* (No. ANI-07) and a sample of *A. marschalliana* (AMA-07) were deposited in the herbarium of the Research Center for Medicinal Plants, Faculty of Chemistry and Chemical Technology, Al-Farabi Kazakh National University, Almaty, Kazakhstan (Figures S31 and S32). The air-dried whole plants of *A. nitrosa* (14 kg) and *A. marschalliana* (13 Kg) were cut into small pieces and stored at room temperature.

2.3. Extraction and Isolation of *A. nitrosa*

The air-dried whole plants of *A. nitrosa* (14 kg) were ground and extracted by 95% aqueous EtOH at room temperature three times (three days each). The EtOH extract was dried with evaporation under reduced pressure. The residue was suspended in H_2O and partitioned with petroleum ether (PE), CHCl_3, and EtOAc (EA), successively (Figure 1). The CHCl_3 fraction (355 g) was subjected to an AB-8 column chromatography (CC), eluted with aqueous EtOH in a gradient manner (30, 50, 70, 80, and 95%), obtaining fractions 1–6. Fraction 2 of 50% of EtOH (110 g) was applied to a polyamide column using H_2O, 50, 70, 95% of EtOH/H_2O (v/v) as an eluent. The water fraction (56 g) was further fractionated over a Sephadex LH-20 column eluted with CHCl_3–MeOH (1:1) to give five fractions (A1–A5). Subfraction A4 was subjected to CC on a ODS medium pressure column eluted with MeOH (through a gradient from 35 to 100% aqueous MeOH) to obtain A4A–A4H + J subfractions. The subfraction A4E (3.9 g) was passed through the silica gel column using a solvent mixture of CHCl_3:MeOH (from 100:1 to 1:1) to give subfractions A4E1–A4E10. Further purification of subfraction A4E was performed by repeated CC over silica gel, giving seven pure compounds, as follows: 1 (110 mg), 2 (54 mg), 3 (23 mg), 4 (17 mg), 5 (38 mg), 6 (7 mg), and 7 (8 mg). Subfraction A4F (602 mg) was purified first by CC over silica gel, and then preparative HPLC (with a gradient of CH_3CN/H_2O) to obtain compounds 8 (3 mg) and 11 (2 mg). In a similar way, subfraction A4C (667 mg) was treated and finally purified by semi-preparative HPLC using CH_3CN/H_2O (15 min, from 50% to 75%, flow rate 3.0 mL/min) to obtain compound 12 (4 mg). Subfraction A4D (3.2 g) was applied to a silica gel column eluted with different solvent systems (CHCl_3:acetone, and CHCl_3:MeOH in a ratio of 100:1 to 10:1) to obtain compounds 9 (1 mg) and 10 (3 mg) (Figure 1).
2.4. Extraction and Isolation of A. marschalliana

The air-dried whole plants of A. marschalliana (13 kg) were powdered and extracted by 95% aqueous EtOH at room temperature three times (3 day each). The EtOH extract was dried with evaporation under reduced pressure. The residue was suspended in water and partitioned with petroleum ether (PE), CHCl₃, and EA, respectively (Figure 2). The obtained CHCl₃ fraction (50 g) was applied to a silica gel column (200–300 mesh) eluted with aqueous EtOH (in a gradient manner from 20% to 95%) to obtain 12 subfractions. The subfractions 7 and 8 (3.579 g) were subjected to CC over Sephadex LH-20 using MeOH as mobile phase, giving five subfractions (7 and 8A to 7 and 8E). Fraction 4 (890 mg) was further applied to CC over silica gel (200–300 mesh) eluting with a gradient solvent system of PE/EtOAc to give compound 1' (11 mg). Subfraction 5 (602 mg) was passed through a column of silica gel (200–300 mesh, PE/EtOAc) to give subfractions 5A–5H. Subsequently, subfraction 5F was purified by preparative HPLC using CH₃CN/H₂O to yield compound 2' (80 mg). Fraction 10 (2.49 g) was subjected to CC over ODS using aqueous MeOH to yield 13 subfractions (Fr10A–Fr10M). Furthermore, subfraction 10J was subjected to CC over Sephadex LH-20 to obtain compound 3' (70 mg). The obtained PE fraction (110 g) was further extracted with 80% aqueous MeOH (34.3 g), which was applied to a polyamide gel column eluted with aqueous EtOH in a gradient manner (20%, 40%, to 95%) to obtain five subfractions (A-E). Subfraction B (3.5 g) was further applied to silica gel CC eluting through a gradient with a solvent mixture of PE/EtOAc (10:1, 1:1, 0:1) to give 10 subfractions (B1–B10). Subfraction B8 (933 mg) was passed through a column of Sephadex LH-20 (eluted with MeOH), giving four subfractions (A to D). Subfraction B8D...
(460 mg) was applied to CC over ODS (aqueous MeOH, from 98:2 (v/v) to 25:75 (v/v)) to obtain subfractions B8D1–B8D5. Subsequently, fraction B8D2 was purified by silica gel CC using a gradient solvent system petroleum ether/EtOAc to obtain compound 4' (7 mg), and then subfractions were subjected to preparative HPLC (CH$_3$CN/H$_2$O) to yield compounds 5' (5 mg), 6' (27 mg), 7' (10 mg), and 8' (3 mg). Subfraction C was treated first by CC over polyamide, and then applied to CC over Sephadex LH-20 eluting with MeOH, obtaining six subfractions (C1 to C6). Subfraction C6 was purified using silica gel CC (CHCl$_3$/EtOAc), obtaining five fractions (C6A to C6E), and then fraction C6E was applied to an Auto-P machine to obtain compounds 9' (1 mg), and compound 10' (8 mg). Subfraction C6C was also applied on an Auto-P machine using CH$_3$CN/H$_2$O as an eluent to obtain compound 11' (3 mg) and compound 12' (2 mg). Fraction C5 was subjected on CC over ODS using CH$_3$CN/H$_2$O as an eluent to obtain compound 13' (2 mg) (Figure 2).

**Figure 2.** Isolation scheme of *A. marshalliana*.

2.5. Cytotoxicity Assay

The cytotoxic effects of *A. nitrosa* and *A. marschalliana* were determined using the colorimetric (CCK8) method [28] and the sulforhodamine B (SRB) protein staining method [29]. The CCK8 method was used to detect the growth inhibition of HL-60 cell lines. Cells with a logarithmic growth phase were seeded into a 96-well culture plate at a specific density (90 µL per well); after culturing overnight, different concentrations of drugs were added
for 72 h. Three replicate wells were set up for each concentration, which corresponds to concentrations of vehicle control and cell-free zero adjustment wells. Then, 10 µL of CCK-8 was added to each well. After incubating for 2–3 h in the incubator, the SpectraMax 190 microplate reader was used to measure the optical density (OD value) at the 450 nm wavelength.

The compound’s inhibitory effect on the proliferation of A549 cells was detected by the sulforhodamine B (SRB) protein staining method. The specific steps are as follows: A549 cells in the logarithmic growth phase are seeded into a 96-well culture plate at an appropriate density, 90 µL per well; after overnight culture, different concentrations of compounds (DMSO concentration less than 0.5%) are added for 72 h, each set has three wells for each concentration, and a solvent control group (negative control) is set. After the effect is over, the culture medium is discarded, and 10% (w/v) trichloroacetic acid (100 µL/well) is added; the solution is fixed at 4 °C for 1 h, then washed with distilled water five times, before being dried at room temperature. Then, we added 100 µL of SRB solution (4 mg/mL, dissolved in 1% glacial acetic acid), incubated it for 15 min at room temperature, rinsed with 1% glacial acetic acid five times to wash away unbound SRB, and added 10 mM Tris solution 100 µL to each well after drying at room temperature, before using a full-wavelength microplate reader SpectraMax 190 at the 515 nm wavelength to determine the OD value.

The inhibitory rate of the compound on cell proliferation is calculated by the following formula: Inhibition Rate = \[1 - \frac{(OD_{cpd} - OD_{untreated})}{(OD_{LPS} - OD_{untreated})}\] * 100%.

2.6. Cell Viability Evaluation

Here, RAW264.7 cells were seeded into 96-well plates at a concentration of 1 × 10^4 cells per well and allowed to adhere to the bottom of the plate overnight. Then, the cells were treated with different concentrations of compounds for 18 h. The cell viability was determined by MTT assay, as described previously [30]. Then, cell viability was determined by incubation with DMEM containing MTT (1 mg·mL^{-1}) for 4 h, followed by dissolving the formazan crystals with 150 µL DMSO. The absorbance at 540 nm was measured by a SpectraMax M5 microplate reader (Molecular Devices, San Jose, CA, USA).

2.7. Measurement of Nitric Oxide (NO) Production

Here, RAW264.7 cells were seeded into 96-well plates (1 × 10^4 cells per well) and allowed to adhere for 24 h. The cells were then treated with different concentrations of compounds or vehicles (DMSO) followed by stimulation with 1 µg·mL^{-1} lipopolysaccharide (LPS, Sigma-Aldrich, St. Louis, MO, USA). The DMSO was used as the vehicle, with the final concentration of DMSO being maintained at 0.1% of all cultures. After 18 h of incubation, the supernatant was collected to determine the NO content using the Griess reagent (Sigma-Aldrich, St. Louis, MO, USA) as described previously [31]. The absorbance at 490 nm was measured by a SpectraMax M5 microplate reader (Molecular Devices, San Jose, CA, USA).

3. Results and Discussion

3.1. Structural Elucidation of Compounds from A. nitrosa

A new germacranolide type sesquiterpenic lactone (11), together with 10 known sesquiterpenic lactones (1–10) and 1 known dimeric sesquiterpenic lactone (12) (Figure 3) were separated from A. nitrosa. After detailed spectroscopic analysis (1D, 2D NMR, LC–MS, TLC) and comparing with the literature data, the known compounds were identified as 1β,9β-Dihydrooxyeudesm-3-en-5a,6β,11β-λ2,6-olide (1) [32,33], decahydro-5,6-dihydroxy-3,5α-dimethyl-9-methylidenaphtho[1,2-β]furan-2(3H)-one (2) [34], deacetylherbolide D (3) [32], deacetyl derivative of herbolide A (4) [32], 1β-ydroperoxy-9β-acetoxygermacra-4,10(14)-dien-6β,11β-12,6-olide (5) [32], herbolide B (6) [32], 11β,13-dihydropodendritin 3-acetate (7) [35], balchanolide (8) [36], 11,13-dihydro germacronolide (9) [37], deacetylherbolide A (10) [38], and artebarrolide (12) [36] (Figures 3 and S1–S14, Supplementary information).
showed m/z 322.087 (Figure S13 Supplementary Material); UV (MeOH) \( \lambda_{\text{max}} \) (log \( \varepsilon \)) 256 (2.07) (Figure S12). The \(^1\)H NMR spectrum data displayed signals of three methyl groups (\( \delta_\text{H} \) 1.58 (d, \( J = 1.3 \) Hz, 3H), 1.27 (d, \( J = 6.9 \) Hz, 3H), and 1.11 (t, \( J = 7.6 \) Hz, 3H)), one exocyclic methylene group (\( \delta_\text{H} \) 4.76, 3.93 each d, \( J = 10.2 \) Hz), and a characteristic signal of a double bond (\( \delta_\text{H} \) 5.13 (dd, \( J = 10.2, 1.6 \) Hz, 1H)) (Table 1). The \(^{13}\)C NMR and DEPT NMR spectra indicated 18 carbon resonances, including 3 methyls (\( \delta_\text{C} \) 17.48, 12.55, 8.53), 5 methylenes (\( \delta_\text{C} \) 37.54, 36.81, 30.76, 27.64, 114.37), 6 methines (\( \delta_\text{C} \) 80.05, 78.81, 74.36, 51.05, 41.56, 121.28), and 4 quaternary carbons (\( \delta_\text{C} \) 177.43, 175.06, 153.51, 145.18) (Table 1). The data suggested that compound 11 might be a germacrane-type of sesquiterpene lactone.

Figure 3. Chemical structures of compounds 1–12 from A. nitrosa.

Compound 11, obtained as a colorless oil, had a molecular formula of C\(_{18}\)H\(_{26}\)O\(_{5}\) on the basis of UV, ESIMS, TOFMS, LC–MS, and NMR spectroscopic data. The TOFMS showed \( m/z \) 322.087 (Figure S13 Supplementary Material); UV (MeOH) \( \lambda_{\text{max}} \) (log \( \varepsilon \)) 256 (2.07) (Figure S12). The \(^1\)H NMR spectrum data displayed signals of three methyl groups (\( \delta_\text{H} \) 1.58 (d, \( J = 1.3 \) Hz, 3H), 1.27 (d, \( J = 6.9 \) Hz, 3H), and 1.11 (t, \( J = 7.6 \) Hz, 3H)), one exocyclic methylene group (\( \delta_\text{H} \) 4.76, 3.93 each d, \( J = 10.2 \) Hz), and a characteristic signal of a double bond (\( \delta_\text{H} \) 5.13 (dd, \( J = 10.2, 1.6 \) Hz, 1H)) (Table 1). The \(^{13}\)C NMR and DEPT NMR spectra indicated 18 carbon resonances, including 3 methyls (\( \delta_\text{C} \) 17.48, 12.55, 8.53), 5 methylenes (\( \delta_\text{C} \) 37.54, 36.81, 30.76, 27.64, 114.37), 6 methines (\( \delta_\text{C} \) 80.05, 78.81, 74.36, 51.05, 41.56, 121.28), and 4 quaternary carbons (\( \delta_\text{C} \) 177.43, 175.06, 153.51, 145.18) (Table 1). The data suggested that compound 11 might be a germacrane-type of sesquiterpene lactone.
The assignments were based on HSQC and HMBC data.

A comparison of NMR data of 11 and the known compound 1β-hydroperoxy-9β-acetoxygermacra-4,10(14)-dien-6β,11β-12,6-olide (5), reported from A. herba-alba [32,33] and also obtained in this study, revealed high similarities between these two compounds, except for an extra methyl group (δH 1.11, m; δC 8.53) (Table 1) present in compound 11. Detailed analysis of the 2D NMR data of 11 further established the structure. The 1H-1H COSY correlations of H-1/H-2/H-3, H-5/H-6/H-7/H-8/H-9, H-7/H-11/H-13, and H-17/H-18 revealed the existence of four segments as shown (Figure 4). The key HMBC correlations from H-1 to C-2 and C-3, H-5 to C-3 and C-15, H-6 to C-8, H-7 to C-13, H-8 to C-7 and C-11, H-14 to C-1, C-9, and H-18 to C-17 further constructed the planar structure of 11, with a propionyloxy group attached to C-9 (Figure 4). The relative configuration of 11 was inferred as the same with that of the known compound 5 by the similar chemical shifts and the similar coupling constants of H-1, H-6, H-9, and H-13 between these two compounds. Therefore, the structure of 11 was fully established, and named 1β-hydroperoxy-9β-propionoxygermacra-4, 10(14)-dien-6β,11β-12,6-olide (11).

Table 1. NMR Data for 11 (500 MHz for 1H and 125 MHz for 13C, in CDCl3, δ in ppm, J in Hz).

| Positions | 1H          | 13C*       |
|-----------|-------------|------------|
| 1         | 3.93 dt (10.5, 1.4) | 74.8       |
| 2         | 2.18–2.04 m   | 31.34      |
| 3         | 2.36–2.21 m   | 37.94      |
| 4         | -            | 153.51     |
| 5         | 5.13 dd (10.2, 1.6) | 121.28     |
| 6         | 4.34 t (9.8)  | 80.05      |
| 7         | 2.36–2.21 m   | 41.56      |
| 8         | 1.97–1.80 m   | 36.81      |
| 9         | 4.76 dt (10.2, 1.5) | 78.81      |
| 10        | -            | 145.18     |
| 11        | 1.97–1.80 m   | 51.05      |
| 12        | -            | 175.06     |
| 13        | 1.27 d (6.9)  | 12.55      |
| 14        | 5.42 d (1.2); 5.34 br s | 114.37     |
| 15        | 1.58 d (1.3)  | 17.48      |
| 16        | -            | 177.43     |
| 17        | 2.36–2.21 m   | 27.64      |
| 18        | 1.11 t (7.6)  | 8.53       |

* The assignments were based on HSQC and HMBC data.

Figure 4. 1H-1H COSY and key HMBC correlations (H→C) of compound 11.
**Compounds 1–10 and 12 have already been isolated and described from other Artemisia species, such as A. herba-alba, A. barrelieri, and A. gyspaea. Artebarrolide (12), which is the first dimeric germacranolide described from A. barrelieri [36], was discovered for the second time in this investigation.**

### 3.2. Structural Elucidation of Compounds from A. marschalliana

A total of 13 compounds were isolated and identified from the whole plant of *A. marschalliana* that grows in Kazakhstan, including coumarins, sesquiterpene diketone, phenylpropanoid, benzopuran derivative, polyacetylenic compounds, fatty acids, naphthalene derivative, flavone, and caffeic acid derivative (Figure 5). By extensive spectroscopic analysis of MS, $^1$H, and $^{13}$C NMR data, and comparison with previously reported data, the structures of known compounds were identified as 1 new 2,2-dimethyl-8-(19-hydroxy)prenyl-6-(12-hydroxy) vinylchromene (1') [39], together with 12 known compounds, namely a methyl 3-(4'′-hydroxyprenyl)-7Z-coumarate (2') [40], arteordoyn A (3') [41,42], 6-acetyl-2,2-dimethylchroman-4-one (4') [43], 5,6,7-trimethoxycoumarin (5') [41], 2-isovaleryl-4-[1-hydroxyethyl]-phenol (6') [44], guayulone (7') [45], diprenylated-dihydroxycinnamic acid (8') [46], dehydrofalcarinol (9') [44], 9,12-Octadecadienoic acid (9Z,12Z)-(2R)-2,3-dihydroxypropyl ester (10') [47], palmarumycin CP 2 (11') [48], 5,7-dihydroxy-6,4'-dimethoxyflavone (12') [49], and propyl caffeate (13') [50] (Figures 5 and S15–S29, Supplementary information).

![Chemical structures of compounds 1'–13' of A. marschalliana.](image-url)
Compound 1', obtained as a colorless oil, had the molecular formula of C_{18}H_{22}O_{3} on the basis analysis of ESIMS and ^{13}C NMR data. The UV spectrum showed maximal absorptions at 239, 274, and 318 nm (Figure S16), indicative of a conjugated aromatic ring. The ^{1}H NMR spectrum showed signals of three methyls (δ_H 1.72 (s, 3H), 1.36 (s, 6H)), two characteristic signals of a double bond (δ_H 5.48 (s, 1H); 6.22 (t, J = 13.6 Hz, 2H)), and a benzol ring. The ^{13}C and DEPT NMR spectra (Table 2) displayed 18 carbon resonances ascribed to 3 methyls (δ_C 27.80, 27.80, 13.37), 2 methylenes (δ_C 27.29, 68.33), 7 methines (δ_C 146.10, 130.60, 129.44, 128.41, 124.09, 123.08, 121.53, 113.92), and 6 quaternary carbons (δ_C 171.21, 152.67, 135.27, 120.60, 125.98, 76.66) (Table 2). The HMBC spectrum revealed H-2 (5.57 (d, J = 9.8 Hz, 1H)) correlated to C-1, C-17, C-18; H-3 (6.22 (t, J = 13.6 Hz, 2H)) to C-8, C-9; correlation in benzoyl ring; moreover H-4 (7.10 (s, 1H)) to C-10; H-6 (6.96 (s, 1H)) to C-10; H-13 (5.48 (s, 1H)) to C-12 and C-14 (Figure 6).

Table 2. NMR Data for 1' (500 MHz for ^{1}H and 125 MHz for ^{13}C, in CDCl_{3}, δ in ppm, J in Hz).

| Positions | ^{1}H | ^{13}C |
|----------|-------|-------|
| 1        | -     | 76.63 |
| 2        | 5.57 (d, J = 9.8 Hz, 1H) | 130.60 |
| 3        | 6.22 (t, J = 13.6 Hz, 2H) | 121.53 |
| 4        | 7.10 (s, 1H) | 129.44 |
| 5        | -     | 128.41 |
| 6        | 6.96 (s, 1H) | 123.08 |
| 7        | -     | 135.27 |
| 8        | -     | 152.67 |
| 9        | -     | 120.60 |
| 10       | 6.22 (t, J = 13.6 Hz, 2H) | 113.92 |
| 11       | 7.58 (d, J = 15.6 Hz, 1H) | 146.23 |
| 12       | 3.25 (d, J = 7.2 Hz, 2H) | 27.29 |
| 13       | 5.48 (s, 1H) | 124.09 |
| 14       | -     | 125.98 |
| 15       | 3.98 (s, 2H) | 68.33 |
| 16       | 1.72 (s, 3H) | 13.37 |
| 17       | 1.36 (s, 6H) | 27.80 |
| 18       | 1.36 (s, 6H) | 27.80 |

* The assignments were based on HSQC and HMBC data.

Figure 6. ^{1}H-^{1}H COSY and key HMBC correlations (H → C) of compound 1’.

The ^{1}H-^{1}H COSY correlations revealed the relations between H-2 to H-3, H-10 to H-11 and H-12 to H-13 (Figure 6). A comparison of compound 1’ with the known compound 2, 2-dimethyl-8-prenyl-6-vinylchromene showed the presence of two hydroxyl groups located at C-12 and C-19 (δ_H 6.22, t; δ_C 114; δ_H 3.98, s; δ_C 68.22) [39]. Accordingly, the full structure of 1’ was proposed and named 2,2-dimethyl-8-(19-hydroxy)prenyl-6-(12-hydroxy) vinylchromene.

Earlier phytochemical studies on A. marschalliana harvested in the Iranian prov-ince of East Azerbaijan led to the isolation and identification of a high concentration of oxygenated sesquiterpenes [26,27], which is surprising due to fewer plants growing in Kazakhstan containing sesquiterpenoid compounds.
3.3. Cytotoxicity Activity

The separated compounds of *A. nitrosa* and *A. marschalliana* were examined for their cytotoxicity against human myeloid leukemia HL-60 cells and A-549 human lung cancer cell lines by the CCK8 and the sulforhodamine B (SRB) protein staining methods, respectively. The results (Tables 3 and 4) showed that monomeric sesquiterpene lactones from *A. nitrosa* showed weak cytotoxic activities against both A-549 and HL-60 cell lines, while the compounds from *A. marschalliana* did not show any effect on the growth of A-549 and HL-60 cell lines (Tables 3 and 4).

Table 3. Cytotoxic activities of isolated compounds from *A. nitrosa* and *A. marschalliana* against the A-549 cell line.

| Compounds of *A. nitrosa* | Inhibition against A-549 (%) | Compounds of *A. marschalliana* | Inhibition against A-549 (%) |
|---------------------------|-----------------------------|---------------------------------|-----------------------------|
|                           | 25 µM | 1 µM   | 20 Mm | 2 µM |
| 2            | 23.7  | 24.8   | <1    | <1   |
| 4            | 28.8  | 30.0   | <1    | <1   |
| 5            | 8.7   | 21.5   | 3.07  | <1   |
| 6            | <1    | <1     | 10.42 | <1   |
| 9            | 22.7  | 24.4   | <1    | <1   |
| 11           | 4.5   | 4.7    | ND    | ND   |
| -            | -     | -      | <1    | <1   |
| ADT          | -     | 84.4   | ADT   | 86.1 |

*ND indicates not determined. ADT indicates positive control.*

Table 4. Cytotoxic activities of isolated compounds from *A. nitrosa* and *A. marschalliana* against the HL-60 cell line.

| Compounds of *A. nitrosa* | Inhibition against HL-60 (%) | Compounds of *A. marschalliana* | Inhibition against HL-60 (%) |
|---------------------------|-----------------------------|---------------------------------|-----------------------------|
|                           | 25 µM | 1 µM   | 20 Mm | 2 µM |
| 5            | 8.7   | 21.5   | 13.04 | <1   |
| 6            | 18.2  | <1     | <1    | <1   |
| 7            | 8.7   | 21.5   | 2.12  | <1   |
| 11           | <1    | 1.9    | <1    | <1   |
| -            | -     | -      | <1    | <1   |
| ADT          | -     | 82.5   | ADT   | 84.0 |

*ND indicates not determined. ADT indicates positive control.*

3.4. Anti-Inflammatory Activity

The sesquiterpenoids isolated from *A. nitrosa* were tested for their inhibitory effects against NO production on LPS-stimulated RAW264.7 macrophages. Firstly, the cytotoxicity of compounds 1–11 was evaluated using the MTT assay to determine the toxicity. Most compounds did not show obvious cytotoxicity towards RAW264.7 cells up to 10 µM (Figure S30). Among the isolates, compounds 2, 9, and 11 showed weak NO inhibitory effects at a concentration of 2.5 µM (Figure S30). Dexamethasone (Dex) was used as the positive control.

4. Conclusions

In this work, a phytochemical study of the whole plants of *A. nitrosa* and *A. marschalliana* growing in Kazakhstan was carried out for the first time. Twelve compounds were purified from *A. nitrosa*, including eight germacranolides, two eudesmanolides, one guaianolide, and one sesquiterpene dimer. Among them, compound 11 is a new germacrene-type sesquiterpene lactone. Moreover, a total of 13 compounds were isolated and identified from *A. marschalliana*, including 1 new chromene derivative (1'), and other known coumarins, sesquiterpene diketone, phenyl propanoid, polyacetylene compounds, fatty acids, naphthalene derivative, flavone, and caffeic acid derivative, respectively. The results revealed the
chemical constituents of these two Artemisia plants of Kazakhstan for the first time. Their chemical constituents differed a lot from each other. The characteristic sesquiterpenoids were disclosed from A. nitrosa, while A. marschalliana was rich in other types of structures rather than sesquiterpenoids. It should be pointed out that the previous investigation of A. marschalliana led to the isolation of rich content of oxygenated sesquiterpenes, which suggested a more in-depth investigation for this species. All the known sesquiterpenes (1–10, 12) have been already reported from the Artemisia species, such as A. herba-alba, A. barrelieri, and A. gypsacea. Artebarrolide (12) is the first dimeric germacranolide reported from A. barrelieri, and it was found for the second time in this study. The biological assay of these compounds is rare in previous investigations.

In this study, the cytotoxicity assay of all isolated compounds and the anti-inflammatory assay of the sesquiterpenoids were performed. The results of the cytotoxicity assay showed that none of these compounds showed significant inhibition against A-549 and HL-60 cell lines. The sesquiterpenoids isolated from A. nitrosa did not show significant inhibition on the LPS-induced NO release from RAW-264.7 cells at the concentrations of 10 and 2.5 μM, which closely correlates to the anti-inflammatory activity. Compared with the compounds isolated from A. heptapotamica in the previous study [24], we found that the sesquiterpenoids obtained from A. nitrosa lack the α,β-unsaturated ketone moiety in their structures, which might be pivotal to the anti-inflammatory activity. It is obvious that more in-depth investigations are needed to discover bioactive compounds from the Artemisia species in Kazakhstan.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules27228074/s1, Supplementary data for Artemisia nitrosa: Figure S1. 1H NMR, 13C NMR, DEPT spectrum of 1 in CDCl3; Figure S2. 1H NMR, 13C NMR, DEPT spectrum of 2 in CD2OD; Figure S3. 1H NMR, 13C NMR, DEPT spectrum of 3 in CDCl3; Figure S4. 1H NMR, 13C NMR, DEPT spectrum of 4 in CDCl3; Figure S5. 1H NMR, 13C NMR, DEPT spectrum of 5 in CDCl3; Figure S6. 1H NMR, 13C NMR, DEPT spectrum of 6 in CDCl3; Figure S7. 1H NMR, 13C NMR, DEPT spectrum of 7 in CDCl3; Figure S8. 1H NMR, 13C NMR, DEPT spectrum of 8 in CDCl3; Figure S9. 1H NMR, 13C NMR, DEPT spectrum of 9 in CD2OD; Figure S10. 1H NMR, 13C NMR, DEPT spectrum of 10 in CDCl3; Figure S11. 1H NMR, 13C NMR, DEPT, HSQC, HMBC and 1H-1H COSY spectra of 11 in CDCl3; Figure S12. UV spectrum of 11 in MeOH. Figure S13. TOFMS spectral analysis of 11. Figure S14. 1H NMR spectrum of 12 in CDCl3. Supplementary data for Artemisia marschalliana: Figure S15. 1H NMR, 13C NMR, DEPT, HSQC, HMBC and 1H-1H COSY spectra of 1’ in CDCl3; Figure S16. UV spectrum of 1’ in MeOH; Figure S17. ESI Mass spectrum of 1’. Figure S18. 1H NMR, 13C NMR, DEPT spectrum of 2’ in Acetone d-6; Figure S19. 1H NMR, 13C NMR, DEPT spectrum of 3’ in CDCl3; Figure S20. 1H NMR, 13C NMR, DEPT spectrum of 4’ in CDCl3; Figure S21. 1H NMR, 13C NMR, DEPT spectrum of 5’ in CDCl3; Figure S22. 1H NMR, 13C NMR, DEPT spectrum of 6’ in CDCl3; Figure S23. 1H NMR, 13C NMR, DEPT spectrum of 7’ in CDCl3; Figure S24. 1H NMR, 13C NMR, DEPT spectrum of 8’ in CDCl3; Figure S25. 1H NMR, 13C NMR, DEPT spectrum of 9’ in CDCl3; Figure S26. 1H NMR, 13C NMR, DEPT spectrum of 10’ in CDCl3; Figure S27. 1H NMR, 13C NMR, DEPT spectrum of 11’ in CDCl3; Figure S28. 1H NMR, 13C NMR, DEPT spectrum of 12’ in CDCl3; Figure S29. 1H NMR, 13C NMR, DEPT spectrum of 13’ in CDCl3. Figure S30. Inhibitory Effects of A. nitrosa compounds on LPS-Enhanced Inflammatory Mediators. Figure S31. Pictures of the whole plant of Artemisia nitrosa. Figure S32. Pictures of the whole plant of Artemisia marschalliana.

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