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Research Article

# Sesquiterpene Lactones and Flavonoids from *Artemisia sieberi*

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**ABSTRACT**
Phytochemical Investigation of the leaves of *Artemisia sieberi* growing in Saudi arabia leads to isolation of eight sesquiterpene lactones of eudesmanolide **1-4**, germacranolide **5-7** and davanone **8** types in addition to six methoxylated flavonoid derivatives **9-14**. The structures of the isolated compounds were elucidated on the basis of spectroscopic evidences and correlated with known compounds. The cytotoxic activity of the isolated compounds was evaluated against human Hela and MCF-7 cell lines. The results exhibited that none of the isolated sesquiterpenoids displaying any cytotoxic activity whilst isolated flavonoids exhibited promising growth inhibitory action.

**Keywords:** *Artemisia sieberi*; Sesquiterpene lactones; Flavonoids; cytotoxicity

## INTRODUCTION

*Artemisia* is one of the largest and most widely distributed genera belonging to the family *Asteraceae*. This genus composed of about 500 diverse species distributed mainly in the temperate zones of Europe, Asia and North America. These species are perennial, biennial and annual herbs or small shrubs[1,2]. Previous phytochemical reports of the genus *Artemisia* reveals that the *Artemisia* species are rich of terpenoids, flavonoids, coumarins, caffeoylquinic acids, sterols and acetylenes compounds[3-7]. Among these bioactive compounds; artemisinin is a highly oxygenated sesquiterpene, containing 1,2,4-trioxane ring which is responsible for its antimalarial activity[8]. Artemisinin exerts not only antimalarial activity but also profound cytotoxicity against tumor cells[9]. *Artemisia* species have a high economic value in several fields, as food plants and in the treatment of many diseases such as hepatitis, cancer, inflammation and infections by fungi, bacteria, and viruses. Furthermore, several species of *Artemisia* are used in folk medicine as anthelminthic, antispasmodic insecticidal, antiatherogenic, hepatoprotective, antihyperglycemic, antihypertensive and in traditional Chinese medicine for the treatment of gynecopathy, amenorrhea, bruise and rheumatic disease[6,10-12]. Although many reports have explored the phytochemical and biological diversity of various species of the genus *Artemisia*, few studies reported on *A. sieberi*. Most of the previous studies focused on the volatile oil composition.[13]. Literature survey showed that *A. sieberi* essential oil contains; α-thujone, β-thujone and camphor with antidiabetic and antimicrobial activity against a large number of microorganisms including Gram positive and negative bacteria, in addition to yeast and filamentous fungi[13-15]. In the course of our ongoing research activities towards the isolation of biologically active compounds from plants growing in Saudi Arabia, either wild or cultivated, in particular the species of diverse chemical constituents with cytotoxic activity, we had the opportunity to work on the leaves of *A. sieberi* to investigate its chemical constituents and their potential biological activities. For *A. sieberi* leaf, there are very few hits describing the leaf extract constituents and activity[15] which encourage us to investigate its active constituents and potential biological activities.

## MATERIALS AND METHODS

*Apparatus and Chemicals*

UV spectra were obtained on a Cary 50 spectrophotometer, Varian, Inc. NMR spectra were recorded at 25 °C with a Varian Inova 400 MHz NMR spectrometer. HRESIMS measurements were acquired with a Thermo scientific LTQ/XL Orbitrap, specifications; analyzer: FTMS,mass range: normal full ms 100-2000, resolution: 30,000. For LC-ESIMS, gradient separation was achieved using a SunFire C-18 analytical HPLC column (5 mm, 4.6×150 mm,Waters) with a mobile phase of 0-100% MeOH over 30 min at a flow rate of 1 mL/min. Column chromatography (CC) was performed using a silica gel (Kieselgel 60 Å, 40-63 µM mesh size, Fluorochem, UK). RP-HPLC were carried

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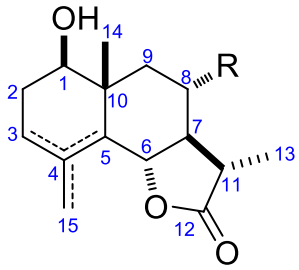
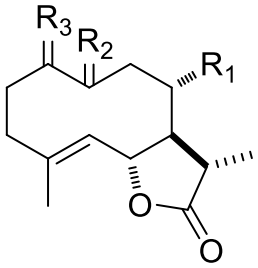
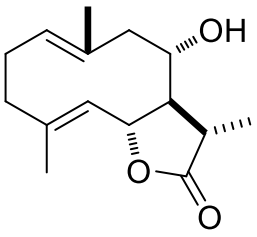


**1** $\Delta^3$, R = OH
**2** $\Delta^4$, R = OH
**3** $\Delta^{4(15)}$, R = H
**4** $\Delta^{4(15)}$, R = OH



**5** $R_1$=H, $R_2$=H, $\beta CH_3$, $R_3$=H, $\beta$OH
**6** $R_1$=OH, $R_2$= $CH_2$, $R_3$= O



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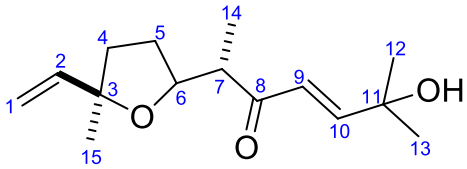
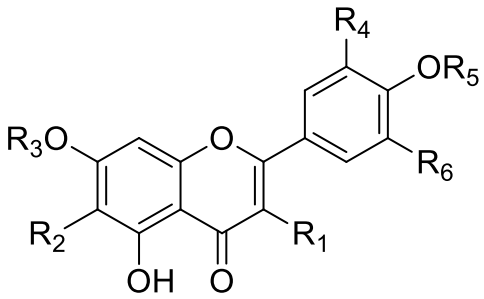


**8**



**9** $R_1$=H, $R_2$=H, $R_3$=$CH_3$, $R_4$=H, $R_5$=H, $R_6$=H
**10** $R_1$=$OCH_3$, $R_2$=H, $R_3$=$CH_3$, $R_4$=H, $R_5$=H, $R_6$=H
**11** $R_1$=OH, $R_2$=H, $R_3$=H, $R_4$=$OCH_3$, $R_5$=H, $R_6$=H
**12** $R_1$=H, $R_2$=$OCH_3$, $R_3$=$CH_3$, $R_4$=OH, $R_5$=$CH_3$, $R_6$=H
**13** $R_1$=H, $R_2$=$OCH_3$, $R_3$=$CH_3$, $R_4$=$OCH_3$, $R_5$=$CH_3$, $R_6$=H
**14** $R_1$=$OCH_3$, $R_2$=H, $R_3$=H, $R_4$=OH, $R_5$=$CH_3$, $R_6$=$OCH_3$

Figure 1: Chemical Structure of the isolated compounds (1-14).

out using Phenomenex Luna C18 (2) (250 × 4.6 mm) (5 $\mu$m) on Shimadzu HPLC-LC-20 AD series binary gradient pump with Shimadzu SPD-M20A detector (Tokyo, Japan). All flash chromatography was performed on Sepacore Flash Chromatography System, Buchi Labortechnik AG, Netherlands. TLC was done using pre-coated silica-gel 60 F254 (0.25 mm, ALUGRAM® SIL G/UV254, Macherey-Nagel, Germany) and RP-18 F254 plates (0.25 mm, Merck, Germany).

*Plant material*

The plant was collected in May 2012 from the western region of Saudi Arabia. The plant material was identified by members of Plant Taxonomy Department, College of Science, Princess Noura bint Abdul Rahaman University. A voucher specimen was deposited at the Herbarium of the Chemistry Department, Science College, Princess Noura bint Abdul Rahaman University (No. ART2012), Riyadh, Saudi Arabia. The leaves were air-dried in the shade, then ground.

*Extraction and isolation*

750 g of the air-dried powdered leaves were extracted by maceration (72 h x 3) with 70% EtOH till complete exhaustion (3L x 3). The alcoholic extract was concentrated and the solvent free residue (62 g, 8.3%) was mixed with 500 mL of distilled $H_2O$, and subjected to successive solvent fractionation with *n*-hexane, chloroform and ethyl acetate till complete exhaustion in each case to give *n*-hexane fraction (13 g), chloroform fraction (22 g), ethyl acetate fraction (8 g) and aqueous fraction (17 g). The chloroform fraction was subjected to flash chromatography on silica gel column using $CHCl_3$–MeOH mixtures in a manner of increasing polarities. Sixty eight fractions (20 mL each) were collected and monitored on TLC (silica gel) using $CHCl_3$–MeOH (90:10), $CHCl_3$–MeOH–$H_2O$ (80:20:2) and (70:30:3) as solvent systems and 10% v/v $H_2SO_4$ in EtOH and/or 0.2% DPPH in MeOH as spraying agents. Similar fractions on TLC were combined to yield 19 groups. The selected groups were subjected to HPLC Phenomenex Luna C18 (2) (250 × 4.6 mm) (5 µm) using a gradient of 5–100% $CH_3CN$–$H_2O$ over 30 min to give 14 compounds. Identified compounds are (6) (13.1 mg) from group 2; (9) (20.8 mg) from group 3; (3) (9.5 mg) from group 5; (5) (7.8 mg) and (7) (10.3 mg) from group 6; (8) (16.5 mg) from group 7; (11) (11.5 mg) from group 8; (13) (12.2 mg) and (14) (8.1 mg) from group 9; (10) (4.6 mg) from group 11; (4) (11.7 mg) from group 14; (1) (6.6 mg) and

Table 1: $^{1}$H NMR spectroscopic data of compounds 1-8 in DMSO-$d_6$ (600 MHz).

| S No. | 1 $\delta^{1}$H/ppm, mult. (*J*) | 2 $\delta^{13}$C/ppm, mult. (*J*) | 3 $\delta^{13}$C/ppm, mult. (*J*) | 4 $\delta^{13}$C/ppm, mult. (*J*) | 5 $\delta^{1}$H/ppm, mult. (*J*) | 6 $\delta^{13}$C/ppm, mult. (*J*) | 7 $\delta^{13}$C/ppm, mult. (*J*) | 8 $\delta^{13}$C/ppm, mult. (*J*) |
|---|---|---|---|---|---|---|---|---|
| 1 | 3.42, m | 3.28, dd (4.8, 11.4) | 3.31, m | 3.77, m | 3.41, m | - | 4.76, m | a. 4.78, d (17.2)<br>b. 4.56, d (17.2) |
| 2 | a. 2.16, m<br>b. 1.80, m | 1.52, m | a. 1.66, m<br>b. 1.38, m | a. 2.09, m<br>b. 1.82, m | a. 2.16, m<br>b. 1.82, m | a. 2.92,m<br>b. 2.21,m | a. 2.07, m<br>b. 2.03, m | 5.52, dd (10.5, 16.7) |
| 3 | 5.28, s | a. 2.19, m<br>b. 1.00, m | a. 2.18, m<br>b. 2.02, m | a. 2.63, m<br>b. 2.47, m | 2.51, m | a. 2.13,m<br>b. 1.95,m | a. 2.19, m<br>b. 1.82, m | - |
| 4 | - | - | - | - | - | - | - | a. 1.47, m<br>b. 1.34, m |
| 5 | 2.16, m | - | 2.07, m | 2.49, m | 5.26, brs | 4.55, d (10.3) | 4.55, m | a. 1.60, m<br>b. 1.19, m |
| 6 | 4.23, t (11.1) | 4.82, m | 4.30, t (11.1) | 4.75, m | 4.20, m | 3.49, m | 4.98, m | 3.72, m |
| 7 | 1.98, m | 2.00, m | 2.06, m | 2.48, m | 1.99, m | 1.46, m | 2.23, m | 2.56, m |
| 8 | 3.71, m | 3.75, m | a. 1.58, m<br>b. 1.42, m | 4.14, m | a. 1.57, m<br>b. 1.47, m | 3.76,m | 3.82, m | - |
| 9 | a. 2.11, m<br>b. 1.02, m | a. 2.08, m<br>b. 1.88, m | a. 1.89, m<br>b. 1.19, m | a. 2.58, m<br>b. 1.53, m | a. 1.85, m<br>b. 1.12, m | a. 2.68,m<br>b. 1.98,m | a. 2.51, m<br>b. 2.16, m | 5.90, d (15.3) |
| 10 | - | - | - | - | 2.14, m | - | - | 6.51, d (15.3) |
| 11 | 2.58, m | 2.62, m | 2.54, m | 2.61, m | 2.50, m | 2.38, m | 2.63, m | - |
| 12 | - | - | - | - | - | - | - | 0.88, s |
| 13 | 1.20, d (7.8) | 1.16, d (7.8) | 1.09, d (7.0 ) | 1.13, d (7.8) | 1.06, d (7.8) | 0.93, d (7.3) | 1.31, d (7.8) | 0.88, s |
| 14 | 0.74, s | 0.98, s | 0.69, s | 1.12, s | 0.74, brs | a. 5.65,s<br>b. 5.56,s | 1.29, s | 0.56, d (7.0) |
| 15 | 1.69, s | 1.73, s | a. 4.85, s<br>b. 4.69, s | a. 5.32, s<br>b. 5.13, s | 1.68, s | 1.22,s | 1.63, s | 0.80, s |

(2) (8.3 mg) from group 16; and finally (12) (8.8 mg) from group 18.

*Cytotoxicity assays*

The *in vitro* cancer growth inhibitory activity of the isolated compounds was determined using MTT colorimetric assay against HeLa (human cervix carcinoma) and MCF-7 (human breast adenocarcinoma) cell lines. MCF-7 and Hela Cells was obtained from ATCC (American Type Culture Collection) and were cultured in Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/l glucose (Sigma–Aldrich), supplemented with 5% fetal bovine serum (Gibco) and 1% of a penicillin–streptomycin mixture (10.000 UI/ml and 10 mg/ml, respectively)[16]. Cells were maintained at 37 $^{o}$C in 5% $CO_2$ and 98% humidity. The cytotoxicity was determined based on the cell viability and measured by the colorimetric MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] reduction assay. The next day, drugs pre-dissolved in DMSO at different concentration were added to the culture. Untreated and blank groups were set as controls. After 48-hour exposure, the MTT solution (5 mg/ml in phosphate buffered saline, PBS) was added to the wells and the plates were incubated for an additional 4 h in a $CO_2$ incubator at 37 $^{o}$C, then the supernatant was discarded, each well was added 150 µL of DMSO. The absorbance was measured at 570 nm in a plate reader (Bio-Tek, USA). The% cell inhibition was determined using the following formula[16,17]:

*Percentage cell inhibition = 100 − Abs (Sample) / Abs (Control) x 100*

## RESULTS AND DISCUSSION

The phytochemical investigations of the leaf extract lead to isolation and identification of eight sesquiterpene lactones of eudesmanolide (**1**-**4**), germacranolide (**5**-**7**) and davanone (**8**) types in addition to six methoxylated flavonoid derivatives (**9**-**14**). (Fig. 1). The eight known sesquiterpene lactones (**1**-**8**) were identified as 1β,8α-dihydroxyeudesm-3-en-6β,7α,11βH-12,6-olide (**1**)[18,19], 1β,8α-dihydroxyeudesm-4-en-6β,7α,11βH-12,6-olide (**2**)[20], dihydroreynosin (**3**)[21], 8α-hydroxy dihydroreynosin (artapshin) (**4**)[22], 1β-droxyeudesm-4-en-6β,7α,11βH-12,6-olide (**5**)[18], 1,8-Dihydroxy-4,10(14),11(13)-germacratrien-12,6-olide; (1α,4E,6α,8β)-form, 11β,13-Dihydro, 1-ketone (**6**)[18], balchanolide (**7**)[23], 2,6,10-Trimethyl-cis-7,10-oxido-dodeca-3E,11-dien-2-ol-5-one (*cis*-hydroxydavanone) (**8**)[24], apigenin 7-*O*-methyl ether

Table 2: $^{13}C$ NMR spectroscopic data of compounds 1-8 in DMSO-$d_6$ (150 MHz).

| S. No | 1 $\delta^{13}C$/ppm, mult. | 2 $\delta^{13}C$/ppm, mult. | 3 $\delta^{13}C$/ppm, mult. | 4 $\delta^{13}C$/ppm, mult. | 5 $\delta^{13}C$/ppm, mult. | 6 $\delta^{13}C$/ppm, mult. | 7 $\delta^{13}C$/ppm, mult. | 8 $\delta^{13}C$/ppm, mult. |
|---|---|---|---|---|---|---|---|---|
| 1 | 73.4, d | 75.8, d | 76.2, d | 76.3, d | 73.3, d | 203.0, s | 127.5, d | 111.2, t |
| 2 | 32.6, t | 26.7, t | 31.2, t | 31.0, t | 32.6, t | 36.9, t | 25.5, t | 144.9, d |
| 3 | 121.9, d | 48.5, t | 33.3, t | 33.3, t | 37.6, t | 35.6, t | 38.7, t | 82.4, s |
| 4 | 132.7, s | 124.5, s | 144.9, s | 144.4, s | 133.1, s | 132.0, s | 138.4, s | 37.1, t |
| 5 | 49.9, d | 129.6, s | 52.4, d | 51.8, d | 121.7, d | 134.0, d | 128.1, d | 29.2, t |
| 6 | 78.4, d | 79.1, d | 78.0, d | 76.4, s | 80.0, d | 77.5, d | 76.4, d | 80.4, d |
| 7 | 55.8, d | 54.6, d | 47.2, d | 54.4, d | 48.6, d | 55.4, d | 54.9, d | 48.5, d |
| 8 | 63.0,d | 63.5,d | 19.8, t | 63.1, d | 19.7, t | 70.0, d | 66.7, d | 202.4, s |
| 9 | 44.8, t | 33.2, t | 35.9, t | 46.3, t | 34.5, t | 39.9,t | 51.3, t | 124.8, d |
| 10 | 39.9, s | 40.7, s | 42.5, s | 42.0, s | 50.5, d | 145.0,s | 135.0, s | 154.3, d |
| 11 | 36.4, d | 36.5, d | 38.1, d | 37.0, d | 39.9, d | 40.5,d | 39.9, d | 69.2, s |
| 12 | 179.0,s | 179.6,s | 179.8,s | 179.7,s | 179.7, s | 179, s | 179.5, s | 29.1, q |
| 13 | 9.1,q | 9.0,q | 9.3,q | 9.0,q | 9.3, q | 9.3,q | 10.6, q | 29.1, q |
| 14 | 12.2,q | 19.6,q | 11.6,q | 12.8,q | 11.0, q | 125.0,t | 16.7, q | 13.4, q |
| 15 | 23.2, q | 19.4, q | 108.9, t | 109.0, t | 23.3, q | 15.6,q | 17.1, q | 26.3, q |

Table 3: Cytotoxic activity of isolated compounds (1-14) against different cell lines.

| Compound/extracts | $IC_{50}$ ($\mu$M) | |
|---|---|---|
| | Hela | MCF-7 |
| 1 | inactive | inactive |
| 2 | inactive | inactive |
| 3 | inactive | inactive |
| 4 | inactive | inactive |
| 5 | inactive | inactive |
| 6 | >100 | >100 |
| 7 | >100 | >100 |
| 8 | inactive | inactive |
| 9 | 23.6 | 47.2 |
| 10 | 18. 3 | 55.4 |
| 11 | 63.2 | 91.9 |
| 12 | 12.5 | 10.3 |
| 13 | 8.1 | 14.7 |
| 14 | 13.8 | 22.1 |
| Doxorubicin | 0.75 | 1.28 |

(puddumetin or genkwanin) (**9**)[25], kaempferol 3,7-*O*-dimethyl ether (kamatakenin) (**10**)[26], quercetin 3'-methyl ether (isorhamnetol) (**11**)[27], 5,3'-dihydroxy-6,7,4'-trimethoxyflavone (eupatorin) (**12**)[28], 5-hydroxy-6,7,3',4'-tetramethoxyflavone (3'-*O*-methyleupatorin) (**13**)[29] and myricetin 3,3',4'-trimethyl ether (**14**)[30]. All physical and spectral data (Table 2) of these compounds were in agreement with the respective published data. All of the isolated compounds from *A. sieberi* were evaluated for their antiproliferative effects using MTT colorimetric assay[31,32] on human Hela and MCF-7 (breast) cell lines using doxorubicin as a positive control. As shown in Table 3, the isolated active flavonoids exhibited more cytotoxicity than sesquiterpenoids. The highest inhibitory activity was observed for compound **12** (eupatorin) against MCF-7 and **13** (3'-*O*-methyleupatorin) against Hela cell lines. Flavonoids have reported growth inhibitory effects on different kinds of cancer cells. Flavonoids can penetrate *in vitro* cultured cells, and modulate the cellular metabolic activities in addition to inhibition of proliferation, reduction of oxidative damage, inactivation of carcinogens, induction of cell cycle arrest and apoptosis, promotion of differentiation, impairment of tumor angiogenesis, and suppression of metastasis[33-39].

## CONCLUSIONS

In this paper, 14 compounds, including 8 sesquiterpene lactones and 6 methoxylated flavonoids, were isolated from the leaf extracts of *A. sieberi*, in which all of them were recorded for the first time. The structural determination was accomplished by the 1D- and 2D-NMR spectra as well as HRESIMS analysis. The isolated compounds were evaluated for their in vitro growth inhibitory activity against different cell lines. Flavonoids free aglycones (**9**-1**4**) showed promising cytotoxic activity and the highest activity was observed with compound **12** on MCF-7 and **13** on Hela cell line.

## ACKNOWLEDGMENT

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