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Two new bioactive salsolanol and biphenylsalsinol from the aerial parts of *Salsola villosa* Delile. ex Schul. (Chenopodiaceae) growing in Saudi Arabia

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ABSTRACT

Objective: To isolate and characterize the bioactive secondary metabolites from aerial parts of widespread Chenopodiaceae taxa growing in Saudi Arabia: *Salsola villosa* Delile. ex Schul.

Methods: Antibacterial activities of chloroformic extract, fractions and isolate compounds was evaluated against five bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Staphylococcus epidermidis*), using a paper disc diffusion method. The purification of compound(s) of chloroform extract was done by chromatographic column of silica gel. The structure elucidation was determined by extensive spectroscopic analysis (^{1}H and ^{13}C nuclear magnetic resonance, correlation spectroscopy, heteronuclear multiple bond correlation, heteronuclear multiple quantum coherence and nuclear overhauser enhancement spectroscopy) and high resolution electrospray ionization mass spectroscopy analysis.

Results: Bioactivity guided fractionation of the chloroformic extract led to the isolation of two bioactive compounds: 4-(4′-hydroxy-2′-methylcyclopent-2′-enyloxy)-4-methylcyclopent-2-enol (1) named salsolanol and 4′-[3-(hydroxymethyl)oxiran-2-yl]-3-[(E)-3-hydroxyprop-1-en-1-yl]-6, 2′-dimethoxy [1, 1′-biphenyl]-2-ol (2) named biphenylsalsinol. The antibacterial effects of the chloroform extracts, fractions and isolated compounds 1 and 2 were also evaluated in this work. Results showed that the compounds 1 and 2 exhibited antibacterial activities against four strains: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa* with diameter of zone of inhibition ranging between (9.33 ± 0.94) to (26.33 ± 0.94) mm.

Conclusions: Based on data presented here, two new natural compounds secondary cyclic alcohol 1 and biphenylpropanoid 2 isolated from bioactive chloroformic extract from aerial parts of *Salsola villosa* can be responsible for its antibacterial activities.

1. Introduction

Salsola is the largest genus of the family Chenopodiaceae and includes over 200 species distributed in arid and semi-arid regions of Middle East, Asia, Europe, and Africa [1–3]. A wide range of structurally diverse secondary metabolites have been identified in *Salsola* species, such as alkaloids (salsolin and salsolidine) that have been isolated from *Salsola kali* [4], flavonoids and phenolic compounds from *Salsola kali*, *Salsola soda*, *Salsola oppositifolia* and *Salsola collina* [5,6], triterpene saponins from *Salsola imbricate*, *Salsola baryosma* and *Salsola somalensis* [7,8], antioxidant bibenzyl and isoflavonoid from *Salsola tetrandra* [9]. Our previous work on the aerial parts of *Salsola tetrandra* leds to new norisoprenoids, long chain fatty hydroxyl, taxiphyllin, trans-N-feruloyltyramine, S-

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(−)-trans-N-feruloyloctopamine and coumarinolignan [10]. In our efforts to discover new and potentially bioactive secondary metabolites from *Salsola* species, we investigated the chloroformic extract of the aerial parts of *Salsola villosa* (*S. villosa*) which grows in Saudi Arabia. Here we report the isolation, the structure elucidation, and the biological activities of two new bioactive compounds. Their structures were elucidated by extensive spectroscopic methods including one-dimensional nuclear magnetic resonance (1D-NMR) and two-dimensional nuclear magnetic resonance (2D-NMR) experiments as well as high resolution electrospray ionization mass spectroscopy (HRESIMS) analysis.

2. Materials and methods

2.1. General experimental procedures

The optical rotations were recorded on a Perkin–Elmer 241-MC polarimeter. Fourier transform infrared spectroscopy (FTIR) spectra were recorded using Perkin–Elmer IR 157G infrared spectrophotometer. ^{1}H-(300 MHz), ^{13}C-(75 MHz) and 2D-NMR spectra of compounds 1 and 2 were recorded in $CDCl_3$ and CD_3OD respectively with a Bruker NMR-300 spectrometer. The residual solvent resonances were used as internal references. Chemical shifts were expressed in ppm and coupling constants were given in Hertz. HRESIMS were measured on a Shimadzu LCMS-IT-TOF mass spectrometry.

2.2. Plant material

Aerial parts of *S. villosa* Delile. ex Schul were collected from Arar, Saudi Arabia, on November 2013. The plant was identified by Dr. Ahmed Kamel Osman, College of Sciences, Department of Biology, Kingdom of Saudi Arabia. A voucher specimen was deposited at the herbarium.

2.3. Extraction and isolation

The air-dried powdered plant 1.5 kg was extracted with methanol at room temperature for 6 days. Evaporation of the solvent under reduced pressure from the crude extract yielded a residue of 164 g. The residue suspended in a H_2O solution (2 L) and then extracted successively with petroleum ether, $CHCl_3$, ethyl acetate (EtOAc) and BuOH, yielding 45, 12.4, 32.4 and 24 g sub-extracts, respectively. Bioactivity-guided fractionation of the chloroformic extract on a silica gel column (mesh 70–230, 70 × 5 cm, inner diamete, *n*-hexane, EtOAc, methanol gradients) led to eight fractions (F_1–F_8). Bioactivity was detected only in fraction F_6 and F_8. The highest antimicrobial effect of sub-fraction F_8 (m = 1.7 g) was rechromatographed by silica gel column using $CHCl_3$/EtOAc gradient as eluent to give five sub-fractions (f_1–f_5). The sub-fraction f_3 (85 mg) which was purified by silica gel column (mesh 70–230, 40 × 1 cm, inner diameter) eluted with $CHCl_3$-EtOAc (80: 20) to yield 6 mg of a bioactive compound 1 (R_f = 0.29, CH_3Cl_2/EtOAc 7:3). The sub-fraction f_5 (56 mg) was purified by preparative thin layer chromatography 85:15 ($CHCl_3$/MeOH) to yield 8 mg of a bioactive compound 2 (R_f = 0.33, $CHCl_3$/MeOH, 9/1). The structures of compounds 1 and 2 were elucidated on the basis of extensive spectroscopic procedures including infrared, high resolution mass spectrometry (HR-MS) and one-dimensional nuclear magnetic resonance (1D-NMR) and 2D-NMR [correlation spectroscopy (COSY), heteronuclear multiple bond correlation (HMBC), and nuclear overhauser enhancement spectroscopy (NOESY)] experiments.

2.4. Antibacterial activities

2.4.1. Bacterial strains

The crude extract, fractions and pure compounds were tested with five reference bacteria at the concentration 1 mg/mL against Gram positive strains represented by *Staphylococcus aureus* ATCC 25923 (*S. aureus*) and *Staphylococcus epidermidis* NCIMB 8853 (*S. epidermidis*) and Gram negative represented by *Escherichia coli* ATCC 25922 (*E. coli*), *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*), *Salmonella typhimurium* ATCC 19430 (*S. typhimurium*).

2.4.2. Preparation of inoculum

Mueller-Hinton (M–H) broth was inoculated aseptically with the appropriate microorganism, 24 h before testing. This was to ensure that the bacteria was fully adapted to the broth and reached the stationary phase of growth. The inoculated bacterial strains were incubated at 37 °C during 18–24 h in Mueller-Hinton agar, the inoculums suspension contain approximately 10^5 CFU/mL colonies.

2.4.3. Disc diffusion method

The antibacterial assay of crude extract and fractions from *S. villosa* was carried out by the paper disc diffusion method [11–14]. A suspension of each tested microorganism (500 µL) was spread on Petri dishes containing specific sterile Mueller-Hinton agar (pH 7.2) cooled medium (DIFCO Muller Hinton agar, lot 1303004, code 0252-17, autoclave at 121 °C for 15 min). Paper discs (6 mm diameter) were impregnated with 20 µL of the crude extract, fractions and pure compounds kept for drying, and placed on the inoculated Petri dishes, which were, after staying at 4 °C for 2 h, incubated at 37 °C for 24 h. The levofloxacin was used as positive control at 5 µg/disc. The developing inhibition zones were measured in millimeters and compared with those of control discs. All tests were performed in triplicate.

3. Results

The antibacterial activities of the chloroformic extract of *S. villosa* aerial parts were evaluated, at the concentration of 1 mg/mL against five bacterial strains (Table 1). The chloroformic extract exhibited activity against four species *S. aureus*, *S. epidermidis*, *E. coli* and *P. aeruginosa* showed an inhibition zone ranging between (10.33 ± 0.81) to (25.33 ± 0.94) mm (Table 1). Bioactivity guided fractionation of the biologically active crude extract by silica gel chromatography led to the isolation of two new antibacterial natural compounds 1 and 2 (Figure 1). 4-(4′-hydroxy-2′-methylcyclopent-2′-enyloxy)-4-methylcyclopent-2-enol (compound 1) named salsolanol and 4′-[3-(hydroxymethyl) oxiran-2-yl]-3-[(*E*)-3-hydroxyprop-1-en-1-yl]-6, 2′-dimethoxy [1, 1′-biphenyl]-2-ol (compound 2) named biphenylsalsinol. 1D-NMR is present in Tables 2 and 3 and 2D-NMR (COSY, HMBC, and NOESY) experiments are showed in Figures 2 and 3.

According to the results given in Table 1, the chloroform extract and fractions (F_6 and F_8) were found to be active towards