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A New Cytotoxic Chromen-C-Glucoside from the Leaves of *Salvadora persica*

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ABSTRACT

The phytochemical investigation of the methanolic extract of the leaves of Salvadora persica afforded five known compounds identified as kampferol-3-O-β-glucopyranoside, Kaempferol-3,7-di-O-β-glucopyranoside, quercetin-3',7-dimethylether, isobiflorin (5,7–dihydroxy-2-methylchromone-8-C-β-glucopyranoside), biflorin (5,7–dihydroxy-2-methylchromone-6-C-β-glucopyranoside), as well as a new cytotoxic chromen derivative; 5-hydroxy-7-methoxy-2-methyl-4H-chromen-4-one-6-C-glucopyranoside. The structure characterization of the isolated compounds was based on MS and NMR spectral data. The cytotoxic activity of the new chromen glycoside was investigated against several cancer cell lines such as Hep-G2, MCF-7, MDA-MB-231 and HT-29. The compound exhibited a cytotoxic activity against Hep-G2, whereas the activity was not significant against MCF-7, MDA-MB-231 and HT-29 cells.

Key words: *Salvadora persica,Chromen-C-glycoside, Cytotoxicity, NMR*

INTRODUCTION

Salvadora persica L. known as miswak is a shrub has a soft white wood and belongs to the Salvadoraceae family. It distributed mainly in tropical and sub tropical Asia. It has been used commonly as toothbrush to strengthen the gums. The fresh root barks and leaves have been used in folk medicine for the treatment of a wide range of medical problems and liver diseases.

The phytochemical investigation of the stem of *Salvadora persica* resulted in the isolation of butanediamide, N1, N4-bis(phenylmethyl)-2(S)-hydroxy-butanediamide, N-benzyl-2-phenylacetamide, N-benzylbenzamide and benzylurea[1]. A new indole alkaloid named salvadoricine was isolated from the same plant species[2]. Five glycosides were isolated from stems of *S. persica* and identified as sodium-1-*O*-benzyl-β-D-glucopyranoside-2-sulphate, 5,5′-dimethoxylariciresinol-4,4′bis-*O*-β-D-glucopyranoside , syringin, liriodendrin and sitosterol-3-*O*-glucopyranoside[3].

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GC-MS analysis of the volatile oil extracted from *Salvadora persica* leaves led to the identification of benzyl nitrile, eugenol, thymol, isothymol, eucalyptol, isoterpinolene, and beta-caryophyllene. Toxicity of the aroma was evaluated using brine shrimp lethality test which gave an LC50 > 1,000 ppm. Using Disc Diffusion Test, it was found that the extract of the leaves has a considerable antibacterial effect on several different oral aerobic bacteria with comparable results to known antibiotics. The extract can be used effectively as a natural tool for teeth cleaning and as a natural analgesic for toothaches[4].

The antiulcer activity of *S. persica* decoction was confirmed using optical microscopy. The elements of gastric mucosa tended to be reestablished normally in treated rats[5].

The antimicrobial activity of aqueous extract of *Salvadora persica* chewing stick's was studied at various concentrations. The extract was effective at lower concentrations against *Strept faecalis*[6]. The antibacterial effect of *Salvadora persica* suggests the presence of volatile active antibacterial compounds[7].

The cytotoxicity of *Salvadora persica* on cultured human and mouse cell lines was evaluated. The results indicated that *S. persica* is toxic to macrophage, epithelial, fibroblast, and osteoblast cells in a concentration-dependent manner[8].

We report here on the isolation and structural characterization of five known phenolic compounds and one new chromen C-glycoside from the methanolic extract of the leaves of *Salvadora persica*. The chemical structures of the isolated compounds were confirmed by extensive UV analysis, ESI-MS and NMR spectroscopic data as well as comparsion with respective literature data. Furthermore, the cytotoxic activity of the new chromen-C-glycoside was also evaluated.

MATERIALS AND METHODS

Plant Material

Salvadora persica leaves were collected from Gabal Fiafa in Jazan, Saudi Arabia on June 2015 and identified by Dr. Wael Kassim, Department of Botany, Jazan University, Saudi Arabia. A specimen was deposited at Jazan University herbarium.

Extraction and isolation

The dried, powdered leaves of *Salvadora persica* (500 g) were extracted with hot MeOH (6 L). The combined extracts were concentrated under reduced pressure to yield a dark gum (37.8 g). The methanol extract was suspended in water and partitioned with hexane, ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH), respectively. The resulting fractions were concentrated *in vacuo* to give a hexane-soluble fraction (2.1 g), an EtOAc-soluble fraction (3.5 g), and a BuOH-soluble fraction (19.9 g). The BuOH fraction was applied to a silica gel column chromatography using CH_2Cl_2/MeOH 10:1 to afford compounds **1-5**.

The ethyl acetate fraction was subjected to silica gel column chromatography using eluent of hexane: ethyl acetate mixtures of increasing polarity to yield six fractions. Compound **6** (42 mg) was isolated and purified from fraction 5 using Sephadex LH-20 column with MeOH.

5-hydroxy-7-methoxy-2-methyl-4H-chromen-4-one-6-C-glucopyranoside (6)

Amorphous white powder. UVmax (MeOH): 278 nm.

ESI-MS: m/z: 369 $[M+1]^+$.

^{1}H- and ^{13}C-NMR (500 MHz, DMSO): Table 1.

Cell culture

Human cancer cell lines HepG2, MCF-7, MDA-MB231 and HT-29 were procured from Korean Cell Line Bank, Korea. The cancer cell lines were grown in DMEM supplemented with 1% antibiotics and 10% FBS at 37°C in a humidified atmosphere containing 5% CO_2. ADSCs were cultured in MesenPRO RS™ Medium supplemented with growth factors (Life Technologies) at the same atmosphere with 5% CO_2.

Antiproliferative assay

The cytotoxic effect of a new chromen-C-glucoside was evaluated by MTT assay method[13]. Briefly, cells were seeded in 96 well plate at a density of 5000 cells/well in 100 µl culture medium. Following 24 h incubation,

cells were treated with various concentrations of **6** and then incubated for 24 h at 37°C with 5% CO_2. After incubation, medium was replaced with 100 µl of MTT solution prepared fresh as 0.5 mg/ml in DMEM, filtered through a 0.22-µm filter, was added to each well, and the plates were incubated in the dark for 4 h at 37°C. Then, the media were removed and 100 µl of DMSO was added to each well and absorbance was measured at 570 nm using a microplate reader. The results are expressed as the percentage of cell viability in comparison with the control cells (Cells without compound). The cell viability of the control group without exposure to the compounds was defined as 100%.

RESULTS AND DISCUSSION

The phytochemical investigation of the methanolic extract afforded five know phenolic compounds (**1-5**) which were identified by different spectroscopic methods as well as comparison with literature data as kampferol-3-*O*-*β*-glucopyranoside[9], Kaempferol-3,7-di-*O*-*β*-glucopyranoside[9], quercetin-3',7-dimethylether[10,11], isobiflorin (5,7–dihydroxy-2-methylchromone-8-*C*-*β*-glucopyranoside)[12], and biflorin (5,7–dihydroxy-2-methylchromone-6-*C*-*β*-glucopyranoside)[12].

Compound **6** was obtained as an amorphous white powder. Its molecular formula $C_{17}H_{20}O_9$ was established by positive-ion HR-ESIMS. The spectrum displayed the molecular ion peak $[M+1]^+$ at *m/z* 369.3701 in agreement with the molecular formula $C_{17}H_{21}O_9$ (calcd. 369.3701).The IR spectrum of **6** showed absorption bands typical of hydroxyl group (3378 cm^{-1}), carbonyl group (1710 cm^{-1}) and aromatic rings (1633, 1600 and 1518 cm^{-1}).

The results of the UV analysis using various shift reagents were in good agreement with the substitution pattern depicted in the suggested structure[12].

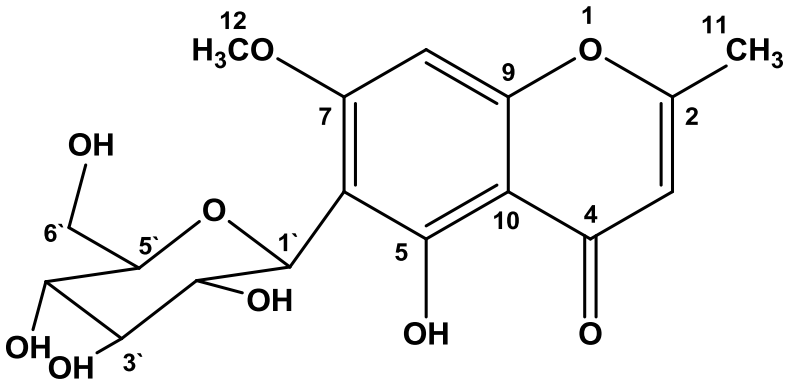

Fig. 1: Compound 6 isolated from the leaves of *Salvadora persica*

The identity of **6** was confirmed unambiguously by a series of 1D and 2D NMR spectral data, including ^1H and ^{13}C-NMR, DEPT, COSY, HSQC and HMBC. The ^1H- and ^{13}C-NMR spectra (including DEPT-135) of **6** showed characteristic shift values and multiplicities of a 6-C-glycosylated chromone derivative[12]. The ^1H NMR spectrum (Table 1) displayed signals at δ 6.17 and 6.37 (each 1H, s) assignable to H-3 and H-8, respectively.

The spectrum further revealed the presence of a methoxyl group at δ 3.87 (3H, s), an isolated methyl proton at δ 2.36 (3H, s), and one anomeric proton signal at δ 4.65 (1H, d, J = 7.6 Hz) for the glucopyranose unit. This was further confirmed by the presence of six carbon signals corresponding to a β-*C*-glucopyranose unit in the ^{13}C NMR and DEPT spectra of **6** (Table 1).

The COSY spectrum and HSQC correlations allowed the assignment of corresponding signals and the remaining NMR signals, as summarized in Table 1.In the ^{13}C NMR spectrum of **6**, the carbon resonances at

δ 74.7 and 108.6 were assigned to the glucosylanomeric carbon and C-6 of the chromone unit, respectively.

In the HMBC spectrum of **6**, the three-bond correlations between the anomeric H-1`at δ 4.67 and both of C-5at δ 160.6 and C-7at δ 163.3 demonstrated that the glucosyl moiety was connected to C-6 of the aglycone unit.It was also proved by the HMBC spectrum that the methoxyl group is attached to C-7, showing correlations between the methoxyl protons at δ 3.87 and both of C-7 at δ 163.3 and C-8 at δ 94.1. The position of the methyl group was assigned to C-2 on the basis of HMBC correlations between C-3 at δ 108.8 and the methyl protons at δ 2.36. The complete assignment of all proton and carbon resonances was achieved after careful analysis of ^{1}H-^{1}H COSY, HSQC and HMBC experiments.

The cytotoxic effect of compound **6** was investigated against Hep-G2 cells. In addition, its comparative activity against the other human cancer cell lines, such as MCF-7, MDA-MB-231 and HT-29 was also examined. The results revealed that compound **6** has a dose-dependent cytotoxic effect against Hep-G2 cells and its dose response curves showed slopes with IC_{50} value of 17.36 µg/mL. whereas the compound does not exhibit any significant cytotoxic effect against MCF-7, MDA-MB-231 and HT-29 cells up to the concentration of 50 µg/ml.

Table 1: NMR data for 6 (500 MHz, DMSO, *J*in Hz, δ in ppm)

Position	δH	δC	HSQC	HMBC (C→H)
2		167.7		
3	6.17 s	108.8	H-3	**3H-11**
4		182.3		**H-3**
5		160.6		
6		108.6		**H-8, H-1`**
7		163.3		**H-8, 3H-12, H-1`**
8	6.37 s	94.1	H-8	
9		156.9		**H-8**
10		104.6		**H-3, H-8**
CH_3	2.36 s	20.5	3H-11	**H-3**
OCH_3	3.87 s	56.2	3H-12	**H-7**
1`	4.65 d (7.6)	74.7	H-1`	
2`	3.21 dd (9.5,7.5)	70.7	H-2`	**H-1`**
3`	3.37 t (9.0)	79.1	H-3`	
4`	3.23 dd (9.5,9.0)	71.1	H-4`	
5`	3.53 m	81.7	H-5`	**H-1`**
6`	3.66 m, 3.50 m	62.3	2H-6`	**H-4**

CONCLUSION

The newly isolated chromen glucoside exhibited promising cytotoxic activity against HepG2 cell lines. Sensitivity to other cancerous cell lines varied according to cell types. Further studies are needed to investigate the mechanism of anti-hepatocarcinomaactivity of the isolated new chromenglucoside.

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