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Original article

Isolation and identification of novel selective antitumor constituents, sidrin and sidroside, from *Zizyphus spina-christi*

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ABSTRACT

Background: The leaves of *Zizyphus spina-christi* (L.) Willd contain several compounds exhibiting different pharmacologic activities. However, studies on the cytotoxic activity of these compounds are limited.
Objectives: We aimed to investigate and isolate cytotoxic compounds with selective antitumor effects from the leaves of *Z. spina-christi* using bioassay-guided fractionation of methanol extract.
Methods: Powdered, dried leaves were subjected to methanol extraction and fractionated using *n*-hexane, chloroform, ethyl acetate, and *n*-butanol. Fractions with positive cytotoxicity against HeLa and THP-1 cell lines were further fractionated and eluted using various concentrations of organic solvents. Active compounds were isolated using different chromatographic methods and their chemical structures were determined using extensive spectroscopic methods, such as 1D NMR (^{1}H NMR, ^{13}C NMR, and DEPT), 2D NMR (COSY, HMBC, and HMQC), HRFAB-MS, and IR. Furthermore, the cytotoxic effects of the isolated compounds were evaluated against 62 tumor cell lines (including HeLa and THP-1) in addition to normal bone marrow cells.
Results: The chloroform and aqueous methanol fractions of the leaves showed cytotoxic activity. Two compounds were successfully isolated and named "sidrin" (13-β-hydroxy-lup-20(30)-ene-2,3-β-epoxy-28-carboxylate) and "sidroside" (3-*O*-β-D-glucopyranosyl-(1–3)-α-L-arabinopyranosyl-jujubogenin-20-*O*-α-L-rhamnopyranoside). Sidrin exhibited cytotoxic activity against the human leukemia (Hl-60, RPMI-8226), lung cancer (A549, EKVX), breast cancer (BT-549, MDA-MB-231/ATCC), colon cancer (KM12), melanoma (M14, SK-MEL-5), and central nervous system (CNS) cancer (SF-295) cell lines, and selectivity was observed against the Hl-60, EKVX, BT-549, KM12, and SF-295 cell lines. In addition, sidrin was more active than sidroside and doxorubicin against the Hl-60 and EKVX cell lines. In contrast, sidrin had a similar effect to doxorubicin against the BT-549 and renal cancer (UO-31) cell lines. Sidroside was more selective against the leukemia (CCRF-CEM, MOLT-4), lung cancer (HOP-92, NCI-H322M), breast cancer (MDA-MB-468), melanoma (LOX IMVI), CNS cancer (SNB-19), ovarian cancer (OVCAR-8), renal cancer (UO-31, RXF 393), and prostate cancer (PC-3) cell lines. Both compounds exhibited similar activity against the breast cancer (MDA-MB-231, T-47D), colon cancer (HCC-2998, HCT-116), ovarian cancer (OVCAR-3), renal cancer (UO-31, 786–0, and SN 12C) cell lines. Normal bone marrow cells were unaffected at the same concentrations of sidrin and sidroside applied to tumor cells.
Conclusions: These results suggest tumor-selective cytotoxicity of sidrin and sidroside.

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1. Introduction

Species of the *Zizyphus* genus (Rhamnaceae) are widespread in tropical American countries, Australia, Africa, and the Mediterranean region. These species are traditionally used in folk medicine because of their antidiabetic, anti-infectious, and sedative properties; moreover, they have been reported to treat various ailments, including fever, dandruff, loss of appetite, bronchitis, pharyngitis, diarrhea, anemia, and insomnia (Abdullah et al., 2022; Marmouzi et al., 2019; Shen et al., 2020; Shergis et al., 2017; Xu et al., 2022). Several compounds, including triterpenoid saponins, tannins, sterols, flavonoids, and cyclopeptide alkaloids, have been isolated and characterized from various *Zizyphus* species (Le Crouéour et al., 2002; Seri et al., 2020).

The subtropical tree *Zizyphus spina-christi* (L.) Willd. is known as "Sidr" or "Nabq" (Ibn Sina [Avicenna], 1025) in Arab countries. This species has been used in folk medicine for different purposes. Its leaves are used for treating abscesses and furuncles, and cataplasms of fresh green leaves are applied to swollen eyes at night. Its leaves are also used as an emollient, a remedy for stomach pains and toothaches, an astringent, and a mouthwash (Duke and Ayensu, 1985). In Saudi Arabian folk medicine, its leaves are used as a sedative and for treating some skin diseases, wounds, various inflammatory conditions, sores, fever, ulcers, diabetes, loss of appetite, infections, insomnia, anemia, and bronchitis (Adzu et al., 2003).

Moreover, the Arabs and Bedouins prepared a paste from the crushed roots, leaves, or branches of *Z. spina-christi* and used it for treating arthritis (Al-Fatimi, 2019; Ghazanfar, 1994). In Arab folk medicine, the leaves of *Z. spina-christi* are also used for cosmetic purposes, such as washing the body and hair (Abu-Rabia, 2015). Furthermore, its leaves are used for treating skin diseases, such as acne and atopic dermatitis, and as an anti-inflammatory, antifungal, and antiseptic agent in Iranian folk medicine (Shakiba et al., 2019).

Previous studies on the leaves, fruits, and barks of *Z. spina-christi* have reported that these plant parts exhibit hypoglycemic, antihyperglycemic (Abdel-Zaher et al., 2005; Glombitza et al., 1994; Nesseem et al., 2009), antidiabetic (Takım, 2021), antidiarrheal (Adly et al., 2022; Adzu et al., 2003; Neamah et al., 2022), antibacterial (El Kutry and Sopeah, 2020), antinociceptive (Adzu et al., 2001; Dhanalekshmi et al., 2022), analgesic, antihyperlipidemic, and antioxidant effects (Adzu and Haruna, 2010; Alsayari and Wahab, 2021; Dkhil et al., 2018; Hussein et al., 2006). In addition, the ethanolic extract of the leaves of this species has been reported to inhibit the growth of *Bacillus subtilis* and exert moderate antipyretic and considerable anti-inflammatory activities (Tanira et al., 1988).

The leaves of *Z. spina-christi* contain several constituent chemicals, such as cyclopeptide alkaloids (Abdel-galil and El-Jissry, 1991; Adam et al., 2022; Tuenter et al., 2017), saponin glycosides, flavonoids (El Maaiden et al., 2019; Hussein, 2019; Pawlowska et al., 2009), and triterpenes (e.g., betulinic and ceanothic acids) (Elghaffar et al., 2022; El-Shahir et al., 2022). Of these constituents, betulinic acid has shown selective cytotoxicity against malignant brain tumors and neuroectodermal and melanoma cells (Wróblewska-Łuczka et al., 2022; Zuco et al., 2002). However, no information is available regarding the tumor-selective cytotoxicity of the other constituents of *Z. spina-christi*.

In a previous study, we discovered and isolated two new biflavonoid ethers, zizyflavosides A and B, from the Egyptian *Z. spina-christi* (Mostafa et al., 2010). Although these compounds failed to show cytotoxicity, we herein conducted further screening for promising antitumor compounds from the same plant leaves using bioassay-guided fractionation of methanol extract. Fractions showing cytotoxicity against HeLa and THP-1 cell lines were subjected to isolation analysis to identify the active compounds, which were further tested against various cancer cell lines compared with normal bone marrow cells.

2. Material and methods

2.1. Instrumentation

The optical rotation of the isolated compounds was detected using a Horiba SEPA-3000 high-sensitivity polarimeter (Horiba, Japan). Ultraviolet (UV) spectra were measured using a Shimadzu UV-1600 UV–Vis spectrometer (Shimadzu, Japan). Infrared (IR) spectra were recorded using KBr disks on a Shimadzu IR-460 IR spectrophotometer (Shimadzu, Japan). Nuclear magnetic resonance (NMR) spectra were obtained using a JEOL GSX-500 spectrometer (JEOL, USA). Chemical shifts were referenced to the residual solvent peaks of the deuterated solvent DMSO d_6 (δ_H 2.49 and δ_C 39.5).

Mass spectra were obtained using a JEOL JMS-SX 102A high-resolution, double-focusing, four-sector tandem mass spectrometer (JEOL, USA). This mass spectrometer was operated at a full accelerating voltage of 10 keV with a standard JEOL FAB source. Sample solutions were poured into the stainless-steel target, and following evaporation of the solvent glycerol (matrix), approximately 3 μL of the matrix was placed on the target and mixed with the sample. The FAB gun, operated with xenon, was used in the ion gun to provide a high primary flux of fast atoms, which was maintained with a 20-mA discharge current at an energy of 5 keV.

Open column chromatography was performed using the following agents: octadecylsilane (ODS) (63–212 μm, Wako Pure Chemical Industries, Ltd., Japan) for reverse-phase chromatography and silica gel (63–210 μm, Kanto Chemical CO., INC, Japan). Reversed-phase-column HPLC was used for further purification of the isolated compounds (Sun-FireTM Prep C18 5 μm; 10 × 250 mm; Waters, USA). Finally, thin-layer chromatography (TLC) was performed using RP-18 F254S and silica gel 60 F254 (Merck, USA) for primary identification of the isolated compounds.

2.2. Plant material

The leaves of *Z. spina-christi* were obtained from a plant cultivated in Ezbet Abuhomoudi, a small village in Qena, Egypt. Plant identification was confirmed by the Department of Botany, Faculty of Science, Assuit University, Egypt. The voucher specimen was coded as T205 and was deposited in the Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assuit Branch, Egypt.

2.3. Extraction and isolation of cytotoxic compounds

Approximately 1.7 kg of the leaves were air-dried and ground to fine powder. This powder was subjected to methanol (CH_3OH) extraction with five sequential macerations, each with two liters of methanol. Each maceration process was performed over 3 days. Then, methanol was evaporated and the residue was redissolved in 1.5 L of 50% aqueous methanol. The redissolved residue was sequentially, repeatedly, and exhaustedly fractionated with *n*-hexane, chloroform ($CHCl_3$), ethyl acetate, and *n*-butanol (0.5 L for each repeated process). After solvent evaporation, dried residues were obtained from each fraction in addition to the remaining aqueous methanol fraction (**supplementary Fig. 1s**).

The dry residue of the aqueous CH_3OH fraction (20 g) was eluted with a mixture of $CHCl_3$/CH_3OH of increasing polarity (9:1,

8:2, 7:3, 6:4, 5:5, 4:6, 3:7, and 2:8) and subjected to column chromatography (CC) on silica gel. The 6:4 fraction that showed the most potent cytotoxic effect was subjected to CC using ODS with water/acetonitrile (H_2O/CH_3CN) as the eluent of increasing polarity (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, and 2:8). The 6:4 fraction again showed the most potent cytotoxic effect. This fraction was purified using HPLC (ODS, 6:4 H_2O/CH_3CN) to yield approximately 20 mg of a novel compound named "sidroside"; this name was derived from the Arabic name of the plant. The $CHCl_3$ fraction (3 g) was subjected to reversed-phase chromatography on ODS with H_2O/CH_3OH as the eluent (polarities: 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, and 2:8). The fraction that showed cytotoxic effect (5:5) was eluted with 50% H_2O/CH_3OH and purified using HPLC (using ODS, H_2O/CH_3OH 6:4) to yield approximately 7 mg of another novel compound named "sidrin.".

2.4. Identification of the sugar moieties of sidroside

Sidroside (4 mg) was dissolved in 3 mL of 5% sulfuric acid (H_2SO_4) in 50% ethanol (CH_3CH_2OH). The mixture was subjected to 100 °C for 3 h to yield sidroside hydrolysate. The latter was extracted with *n*-butanol, purified via silica gel chromatography, and assessed via TLC (silica gel using the solvent system of $CHCl_3$:CH_3OH:H_2O [65:40:10]) against the authentic reference standards of L-arabinose (retention factor [R*f*] = 0.50), L-rhamnose (R*f* = 0.60), and D-glucose (R*f* = 0.41). The TLC procedure was repeated with another solvent system (CH_3CN:H_2O [17:3]) for confirmation (**Supplementary Fig. 2s**). Finally, the sugars were converted to benzoyl derivatives and identified using chiral HPLC, as described in the next section.

2.5. Determination of the stereochemistry of the sugar moieties of sidroside via chiral HPLC

Each sugar moiety of sidroside was dissolved in dry pyridine (C_5H_5N) as 1 mg/0.5 mL, and the solution was ice-cooled. Later, 0.1 mL of benzoyl chloride (C_7H_5ClO) was added, followed by stirring for 35 h at room temperature. Finally, dropwise addition of 0.1 mL of methanol to the reaction mixture was followed by stirring for 30 min. The mixture was diluted with ethyl acetate ($CH_3COOC_2H_5$) and aqueous sodium carbonate (Na_2CO_3). This led to the formation of two layers (organic and aqueous). The organic layer was washed with brine to remove any aqueous contaminants. In contrast, the organic portion in the aqueous layer was extracted with ethyl acetate and added to the washed organic layer. The combined organic extract was dehydrated with magnesium sulfate ($MgSO_4$) and concentrated. The resulting dark brown oil residue was purified using silica gel CC (eluted with *n*-hexane/ethyl acetate [5:1]) to yield the corresponding benzoyl derivative of the sugar. The benzoyl derivative of the sugar moieties of sidroside was subjected to chiral HPLC on a CHIRALPAK IB column (4.6 × 250 mm, methanol, 0.70 mL/min, and UV detection at 254 nm) (Daicel Corporation, Japan). The retention times of the benzoyl sugar derivatives of sidroside were 11.8, 11.4, and 8.2 min. These retention times matched those of the synthetic benzoyl derivatives of D-glucopyranose, L-rhamnopyranose, and L-arabinopyranose, respectively.

2.6. Sidrin and sidroside stock solutions

Sidrin and sidroside were prepared as stock solutions of 100 μM in DMSO. Parts of the stock solutions were diluted to different concentrations ranging from 1 to 100 μM, according to the required test.

2.7. Cytotoxicity of sidrin and sidroside

2.7.1. Cytotoxicity against myeloma THP-1 monocyte cell line

3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay was performed according to the method followed by Niksic et al. (Niksic et al., 2021). Cell suspensions (180 μL) were seeded in 96-well plates at a density of 1.0×10^5 cells per well. Approximately 20 μL of each concentration of sidrin and sidroside was added. The cells were cultured for 3 d, and approximately 10 μL of MTT was added to the cultured cells. They were incubated for 4 h and solubilized in 100 μL of 10% SDS–N,N-dimethylformal dehyde (DMF) solution for 10 h. A microplate reader was used to measure the absorbance at 570 nm.

2.7.2. Cytotoxicity against HeLa cells

Dulbecco's modified Eagle's medium was used for growing cells. This medium was supplemented with streptomycin (50 mg/mL), penicillin (50 units/mL), and 10% fetal calf serum at 37 °C under a humidified atmosphere of 5% CO_2. The cells were seeded into a 96-well microplate (3×10^3 cells/well) and cultured for 24 h. The medium was replaced with a fresh medium containing sidrin and sidroside. The cells were cultured for 3 d at 37 °C. The medium was again replaced with 50 μL of MTT solution (0.2 mg/mL in the medium). The cells were incubated under the same conditions for 4 h after adding 200 μL of DMSO. A microplate reader was used to measure the optical density at 570 nm.

2.7.3. Cytotoxicity against 60 cell lines from the National cancer Institute

This test was performed at the National Cancer Institute (NCI, USA). The test used standard 60 human cancer cell lines including subpanels of melanoma; leukemia; and prostate, breast, kidney, ovary, brain, colon, and lung cancer cell lines (Shoemaker, 2006). These cell lines had reproducible growth and drug sensitivity profiles and were adapted to a single-growth medium (Rubinstein et al., 1990). Doxorubicin was used as the positive control.

2.7.4. Bone marrow cell preparation and colony-forming unit assay

Murine femurs and tibiae were used as the sources of bone marrow, as described by an earlier study (Skehan et al., 1990). The bone marrow was aspirated with a syringe filled with phosphate-buffered saline to obtain the cells. The remaining cells were obtained by washing the syringe twice with the buffer. A hemocytometer was used to count bone marrow cells that were later kept ice-cold in plastic tubes before conducting colony-forming-unit (CFU) assay (Ildstad and Sachs, 1984; Wang et al., 2022b).

2.8. Statistical analysis

The results are expressed as means ± standard errors of the means of at least three independent experiments. Comparisons between the means were performed using one-way analysis of variance allied with Dunnett's post hoc analysis for significance. Differences with $P < 0.05$ were considered statistically significant.

3. Results

3.1. Positive cytotoxic activity of the tested fractions

Only the chloroform and aqueous methanol fractions showed cytotoxicity against the HeLa and THP-1 cell lines (**Supplementary Fig. 1s**).

3.2. Structure elucidation of sidrin

Sidrin, white amorphous powder, showed a quasimolecular ion peak $[M-H]^-$ at *m/z* 483.34784 in HRFAB-MS, which was compatible with a molecular formula of $C_{31}H_{47}O_4$ (**Supplementary Fig. 3s**). Absorption for a carbonyl group (1740 cm^{-1}), a hydroxyl group (3450 cm^{-1}), and a terminal double bond (3070, 1690, and 885 cm^{-1}) were revealed via IR spectroscopy (**Supplementary Fig. 4s**). Signals for five tertiary methyl groups (at δH 0.63, 0.75, 0.85, 0.86, and 0.92), one vinylic methyl group (at δH 1.63), and two olefinic hydrogens (at δH 4.68 and 4.54) of the 29th carbon in addition to signals for one carboxylate (δH, 8.30) were revealed via ^{1}H NMR (Table 1, **Supplementary Fig. 5s**). These signals are characteristic features of a triterpenoid lup-20(30)-ene parent structure (Qiao et al., 2019).

The 31 carbon signals of sidrin were assigned to 23 groups (six methyl, eleven methylene, and six methine groups) in addition to eight quaternary carbons (including one hydroxylated carbon and one carbonyl carbon), as revealed via ^{13}C NMR and DEPT (**Supplementary Fig. 6s–7s**). The presence of one extra ring between C-2 and C-3 was indicated by the HMBC of the correlations between the following: H-2 and C-3; H-1, C-2, and C3; methyl-24 and C-3; and the unsaturation degree. The presence of an epoxide ring was supported by resonances at δc 49.8 (C-2) and δc 76.7 (C-3), associated with δ_H 2.96 (H-3) and δ_H 1.48 (H-2), with a minor coupling constant (**Supplementary Fig. 5s**). In addition, β orientation of the epoxide ring was indicated by NOE correlations of H-3 with H-2 and H-5 with H-9 (**Supplementary Figures 8s–9s**).

The HMBC correlation between both H-12 (at δ_H 1.40) and H-18 (at δ_H 1.46) and C-13 (at δc 79.1) confirmed that the latter is the hydroxylated quaternary carbon. On the other hand, the HMBC correlation between H-28 (at δ_H 1.30) and C-31 (at δc 177.1) confirmed that the latter is the carbonyl carbon, which was assigned as a carboxylate by IR. The proposed stereochemical configuration of the structure was confirmed using the NOE experiment. This NOE experiment corresponds with lupeol biosynthesis (Czarnotta et al., 2017; Valencia-Chan et al., 2022). Based on the above observations, the structure of sidrin was suggested to be 13-β-hydroxy-lup-20(30)-ene-2,3-β-epoxy-28- carboxylate (Figs. 1 and 2 **and Supplementary 10s**).

3.3. Specifications of sidrin

The characteristics of sidrin are as follows: white, amorphous powder; $[\alpha]_D^{24}$ + 8.5° (*c* 0.20, CH_3OH); HRFAB-MS *m/z* 483.34744 $[M-H]^-$ (calculated for $[C_{31}H_{47}O_4]^-$ 483.34784); UV λ_{max} (CH_3-OH) (log ε) 204.5 (4.0) nm; IR ν_{max} (KBr disk) 3450, 1690, 3070, 1740, 1050, and 885 cm^{-1}; ^{1}H NMR, and ^{13}C NMR data (Table 1).

3.4. Structural elucidation of sidroside

Sidroside was obtained as white amorphous powder and tested positive for sugars (Molisch test), triterpenes (Liebermann–Burchard test), and saponins (froth test). Its molecular formula was determined to be $C_{47}H_{77}O_{17}$ based on the quasimolecular ion peak $[M + H]^+$ *m/z* 913.51574 in the positive HRFAB-MS (**Supplementary Figure 11s**). The aglycone was assigned as jujubogenin based on the ^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, HMQC, and HMBC experiments (**Supplementary Figures 12s–17s**). Most ^{13}C NMR signals, except those for the sugars of sidroside, were assigned through $^2J_{H-C}$ and $^3J_{H-C}$ couplings of the seven methyl groups. These groups were similar to those in the literature (Sakna et al., 2022). Signals for seven tertiary methyl groups at δ_H 0.77, 0.89, 1.01, 1.01, 1.23, 1.58, and 1.64 and an isobutenyl group at δ_H 5.09 (1H), 1.58 (3H), and 1.64 (3H) were observed in the ^{1}H NMR spectrum of sidroside (Table 2).

The carbon chemical shifts of the triterpenoid units assigned to C-17, C-20, C-21, C-24, and C-25 corresponded to the values for those of the jujubogenin aglycone (Wang et al., 2022a). The assignments were further confirmed by HMBC, including correlations from one of the methylene protons on C-22 (appearing at δ_H 1.37) with C-17, C-20, C-21, and C-23 and from a methine proton on C-23 (resonating at δ_H 4.49) with C-24 and C-25 (Fig. 3). The presence of three sugars was indicated by three anomeric signals at δ_H 4.29 (d, *J* = 6.8 Hz), 4.31 (d, *J* = 7.8 Hz), and 5.30 (brs). These anomeric signals were correlated with carbon signals at δ_C 104.0, 103.2, and 100.1, respectively. Complete assignment of each sugar proton signal was achieved using ^{1}H–^{1}H COSY correlations. β-glucopyranosyl, α-arbinopyranosyl, and α-rhamnopyranosyl moieties were identified by evaluating coupling constants and chemical shifts (Table 2).

Table 1
NMR data (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR in DMSO d_6) for sidrin.

No	δ_C	δ_H (*J* in Hz)	No.	δ_C	δ_H (*J* in Hz)
1	38.4	1.54 (1H, d, 5.1), 1.58 (1H, m)	17	41.9	–
2	49.8	1.48 (1H, brt, 3.9)	18	48.5	1.46 (1H, dt)
3	76.7	2.96 (1H, d, 2.9)	19	46.5	2.93 (1H, m)
4	40.2	–	20	150.2	–
5	54.8	0.96 (1H, dd, 13.2, 4.4)	21	27.1	1.78 (1H, m), 1.40 (1H, m)
6	17.9	1.44 (1H, m), 1.29 (1H, m)	22	36.4	1.80 (1H, m), 1.45 (1H, m)
7	30.1	1.43 (2H, m)	23	28.1	0.86 (3H, s)
8	36.4	–	24	14.4	0.86 (3H, s)
9	37.8	2.21 (1H, td, 12.2, 12.2, 3.1)	25	15.8	0.75 (3H, s)
10	31.5	–	26	14.1	0.92 (3H, s)
11	20.4	1.43 (1H, m), 1.37 (1H, m)	27	15.8	0.63 (3H, s)
12	31.8	1.40 (1H, m), 1.08 (1H, m)	28	29.1	1.30 (2H, s)
13	79.1	–	29	109.5	4.68 (1H, brs), 4.56 (1H, brs)
14	55.3	–	30	18.9	1.63 (3H, s)
15	25.0	1.43 (1H, m),1.37 (1H, m),	31	177.1	8.30 (1H, s)
16	33.8	1.40 (1H, m), 2.10 (1H, m)			

brs, broad singlet; ***brt***, broad triplet; ***d***, duplet; ***dd***, duplet of duplet; ***dt***, duplet of triplet; ***m***, multiplet; ***s***, singlet; ***td***, triplet of duplet.

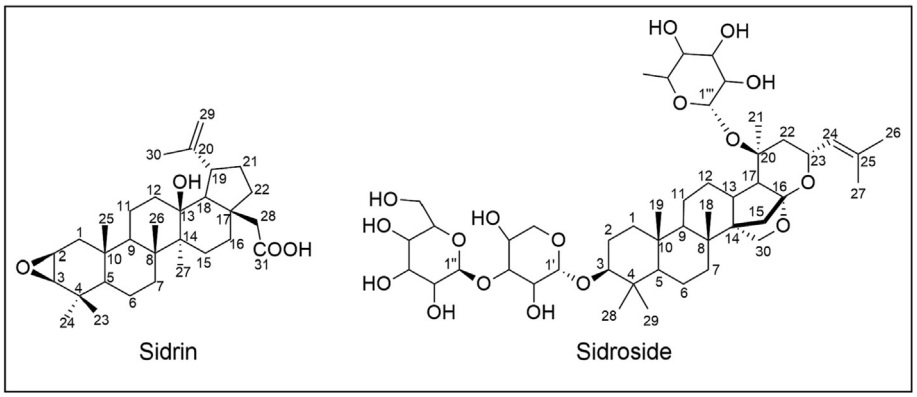

Fig. 1. Chemical structures of sidrin and sidroside.

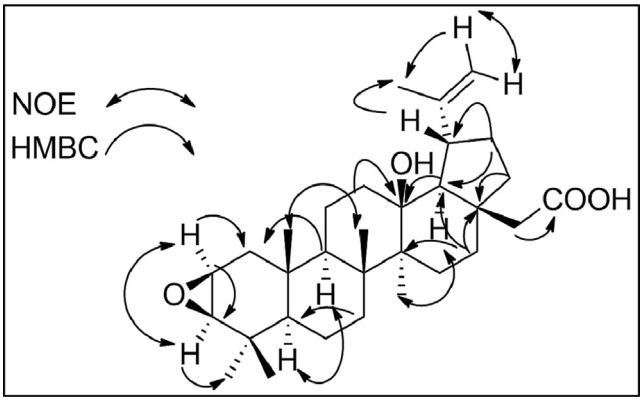

Fig. 2. Key HMBC and NOE correlations of sidrin.

HMBC correlations between signals at δ_H 4.29 (arabinose anomeric proton-1′) and δ_C 87.2 (C-3 of the jujubogenin aglycone) and between signals at δ_H 4.31 (glucose anomeric proton-1″) and δ_C 67.3 (arabinose C-3′) indicated that the diglycosidic chain was β-glucopyranosyl-(1–3)-α-arabinosyl, which was linked to jujubogenin at C-3 (Fig. 3). The linkage of the third sugar unit (α-rhamnose) on the C-20 of jujubogenin was deduced from the HMBC correlation between δ_H 5.30 (rhamnose anomeric proton-1‴) and δ_C 66.3 (C-20 of the jujubogenin aglycone). These linkages were confirmed via NOE correlations between signals of arabinose anomeric proton-1′ and the proton on C-3 of the jujubogenin aglycone, between glucose anomeric proton-1″ and arabinose proton on C-3′, and between rhamnose anomeric proton-1‴ and protons on C-21 of the jujubogenin aglycone. The sugar constituents of sidroside were identified as β-D-glucose, α-L-arabinose, and α-L-rhamnose using co-TLC (against the authentic reference standards

Table 2
NMR data (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR in DMSO d_6) for sidroside.

No.	δ_C	δ_H (*J* in Hz)	No.		δ_C	δ_H (*J* in Hz)
1	38.7	1.53 (1H, m), 0.85 (1H, m)	25		133.4	–
2	25.9	1.51(1H, m), 0.87 (1H, m)	26		25.2	1.64 (3H, s)
3	87.2	3.00 (1H, dd, 11.7, 4.3)	27		18.4	1.58 (3H, s)
4	40.0	–	28		27.1	0.75 (3H, s)
5	55.3	0.75 (1H, m)	29		16.4	0.86 (3H, s)
6	18.0	1.44 (1H, m), 1.58 (1H, m)	30		64.9	3.69 (1H, m), 3.74 (1H, m)
7	35.1	1.45 (1H, m), 1.33 (1H, m)	Arabinose	1′	104.0	4.29 (1H, d, 6.8)
8	38.2	–		2′	76.8	3.10 (1H, d, 6.8)
9	52.5	0.80 (1H, m)		3′	82.3	4.85 (1H, dd, 5.7, 8.2)
10	38.2	–		4′	67.3	3.85 (1H, m)
11	21.0	1.30 (1H, m), 1.52 (1H, m)		5′	64.6	3.64 (1H, dd, 3.0, 12), 3.66 (1H, dd, 2.0, 12)
12	27.6	1.54 (1H, m), 1.73 (1H, m)	Glucose	1″	103.2	4.31 (1H, d, 7.8)
13	35.6	2.38 (1H, m)		2″	73.3	3.03 (1H, dd, 7.5, 9.0)
14	51.9	–		3″	76.7	3.12 (1H, t, 9.0)
15	35.8	1.88 (1H, d, 8.8), 2.38 (1H, m)		4″	69.7	3.40 (1H, t, 9.0)
16	109.3	–		5″	76.8	3.14 (1H, ddd, 2.5, 4.5, 9.0)
17	55.3	1.52 (1H, m)		6″	60.9	3.41 (1H, m), 3.63 (1H, m)
18	18.4	1.01 (3H, s)	Rhamnose	1‴	100.1	5.30 (1H, brs)
19	16.4	0.65 (3H, s)		2‴	70.1	3.60 (1H, m)
20	66.3	–		3‴	67.1	3.86 (1H, m)
21	29.2	1.23 (3H, s)		4‴	72.6	3.70 (1H, m)
22	44.1	1.23 (1H, m), 1.37 (1H, m)		5‴	67.4	4.10 (1H, d, 6.8)
23	57.4	4.49 (1H, t, 9.5)		6‴	16.5	1.04, (3H, d, 6.8)
24	125.8	5.09 (1H, d, 7.8)				

brs, broad singlet; ***d***, duplet; ***dd***, duplet of duplet; ***ddd***, duplet of duplet of duplet; ***m***, multiplet; ***s***, singlet; ***t***, triplet.

of the sugars) and chiral HPLC (after acid hydrolysis of sidroside). Based on these findings, the structure of sidroside was suggested to be 3-*O*-β-D-glucopyranosyl-(1–3)-α-L-arabinopyranosyl-jujubo genin-20-*O*-α-L-rhamnopyranoside, as shown in Fig. 1.

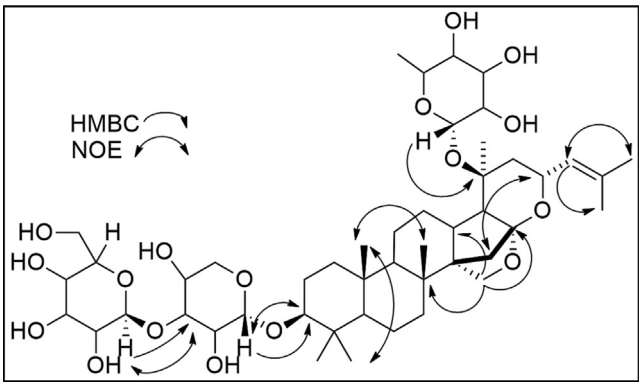

Fig. 3. Key HMBC and NOE correlations of sidroside.

3.5. Specifications of sidroside

The characteristics of sidroside are as follows: white, amorphous powder; $[\alpha]_D^{23}$ – 6° (*c* 0.30, CH_3OH); HRFAB-MS *m/z* 913.51603 $[M + H]^+$ (calculated for $[C_{47}H_{77}O_{17}]^+$ 913.51574); UV λ_{max} (CH_3OH) (log ε) 313 (sh) (3.1), 288 (sh) (3.1) and 205 (4.2) nm; ^1H NMR and ^{13}C NMR data (Table 2).

3.6. Cytotoxicity of the isolated compounds

3.6.1. Myeloma THP-1 monocyte cell line

Both sidrin and sidroside showed inhibitory effect on the myeloma THP-1 monocyte cell line. The observed half-maximal inhibitory concentration (IC_{50}) values were 7.2 and 3.6 μM for sidrin and sidroside, respectively (Fig. 4).

3.6.2. HeLa cell line

Both sidrin and sidroside showed inhibitory effect on the HeLa cell line. The observed IC_{50} values were 5.1 and 2.7 μM for sidrin and sidroside, respectively (Fig. 5).

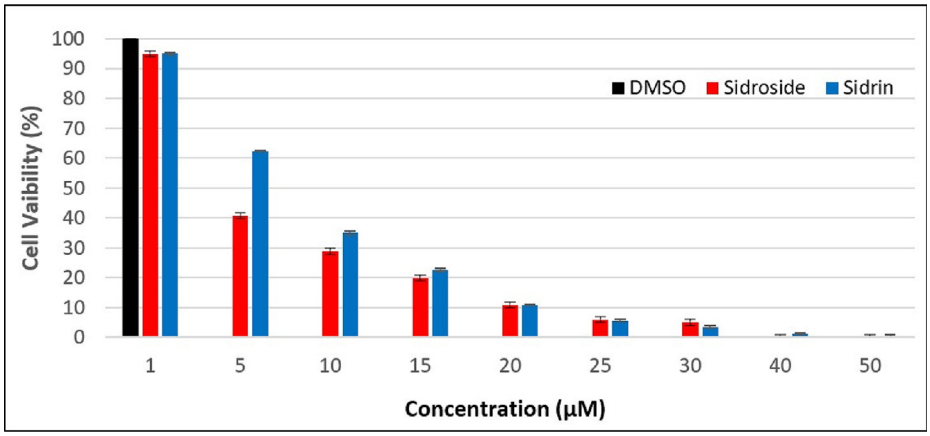

Fig. 4. Cell viability of the myeloma THP-1 cell line after treatment with different concentrations of sidrin and sidroside compared with dimethyl sulfoxide (DMSO) as a control (n = 3, P < 0.001 for both compounds).

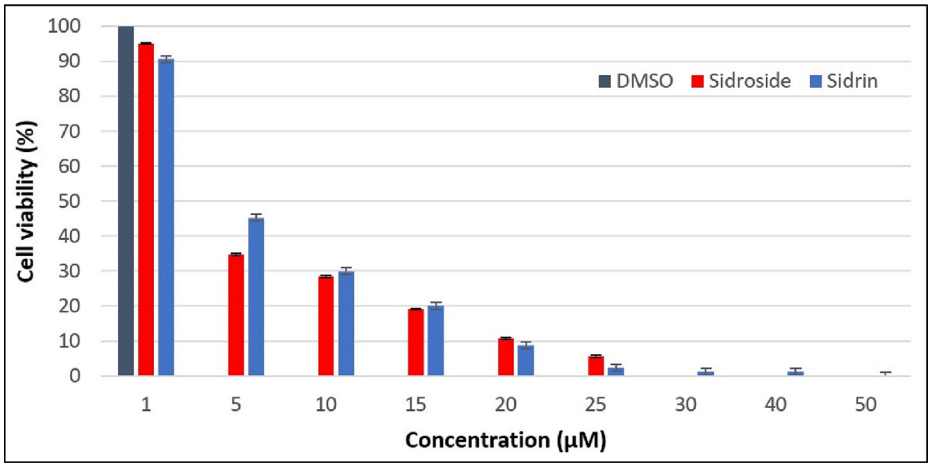

Fig. 5. Cell viability of the HeLa cell line after treatment with different concentrations of sidrin and sidroside compared with dimethyl sulfoxide (DMSO) as a control (n = 3, P < 0.001 for both compounds).

Table 3
Cytotoxicity of sidrin and sidroside against 60 human cancer cell lines performed at the National Cancer Institute, USA.

Cell line	Growth inhibition (%)		
	Sidrin (10 μM)	Sidroside (10 μM)	Doxorubicin (2.5 μM)
Leukemia			
HL-60	27.9	0.0	19.1
K-562	28.1	22.2	71.8
CCRF-CEM	Not evaluated	47.40	46.7
MOLT-4	0.0	39.33	100
RPMI-8226	19.8	24.25	37.3
Non-small cell lung cancer			
A549/ATCC	25.8	11.8	64.5
EKVX	23.6	0.0	11.2
HOP-92	0.0	32.3	49.0
NCI-H322M	0.0	20.9	0.0
NCI-H522	21.9	9.4	4.5
Breast cancer			
BT-549	33.4	0.0	34.4
MDA-MB-231/ATCC	14.9	21.5	17.9
MDA-MB-468	0.0	23.8	37.6
T-47D	23.7	44.3	60.5
Colon cancer			
HCC-2998	15.8	31.1	100
KM12	19.9	0.0	46.3
HCT-116	16.7	23.6	71.9
Melanoma			
M14	13.5	0.0	50.0
LOX IMVI	0.0	17.1	79.4
SK-MEL-5	15.2	0.0	58.1
CNS cancer			
SF-295	14.8	0.0	100
SNB-19	0.0	23.5	64.4
Ovarian cancer			
OVCAR-8	0.0	18.0	55.0
OVCAR-3	11.1	13.6	27.2
Renal cancer			
786–0	12.7	26.6	74.3
RXF 393	0.0	34.9	n.t
SN 12C	16.8	16.4	97.5
UO-31	22.2	29.5	23.7
Prostate cancer			
PC-3	19.5	27.6	25.5

3.6.3. The 60 cell lines from the NCI

Both sidrin and sidroside showed selective cytotoxic activity against different melanoma; leukemia; and prostate, ovarian, renal, breast, lung, CNS, and colon cancer cell lines (Table 3 **and supplementary Tables 1s–2s**).

3.6.4. Bone marrow cells

In the CFU assay, no cytotoxic activity was observed on the cultured murine bone marrow cells for sidrin at 10 and 100 μM concentrations and sidroside at 5 and 50 μM concentrations (Fig. 6).

4. Discussion

Cytostatic activity testing of stem barks of different plants using various *in vivo* cancer model systems has led to the discovery of the antitumor activity of lupane-derived triterpenoids (Hordyjewska et al., 2019). The best-known representative of the lupane-derived compounds with antiproliferative properties is betulinic acid (Hamedi et al., 2022; Siddiqui et al., 2022). Previous studies also showed that cancer apoptosis was induced by topoisomerase I poisons (Abdel Bar et al., 2009; Santana Gomes et al., 2022; Talukdar et al., 2022).

In the present study, the *in vitro* cell proliferation experiment of sidrin and sidroside against the THP-1 cell line showed moderate cytotoxicity of both compounds with an IC_{50} value of 7.2 μM for sidrin and 3.6 μM for sidroside. In addition, both compounds showed cytotoxicity on the HeLa cell line, with IC_{50} at 5.1 μM and 2.7 μM for sidrin and sidroside, respectively. Moreover, sidrin and sidroside showed moderate cytotoxicity against melanoma; leukemia; and prostate, breast, kidney, ovary, brain, colon, and lung cancer cell lines (among the 60 cell lines tested at the NCI) at 10 μM.

Sidrin exhibited antitumor activity against human leukemia (Hl-60 and RPMI-8226), breast cancer (BT-549 and MDA-MB-231/ATCC), lung cancer (A549 and EKVX), colon cancer (KM12), melanoma (M14, SK-MEL-5), and CNS cancer (SF-295) cell lines. However, sidrin showed selectivity against the Hl-60, EKVX, BT-549, KM12, and SF-295 cell lines. In addition, sidrin was more active than sidroside and doxorubicin against the Hl-60 and EKVX cell lines, while it had a similar effect to doxorubicin against the BT-549 and renal cancer (UO-31) cell lines. On the other hand, sidroside was more selective against lung cancer (HOP-92 and NCI-H322M), leukemia cancer (MOLT-4 and CCRF-CEM), breast cancer (MDA-MB-468), melanoma (LOX IMVI), CNS cancer (SNB-19), prostate cancer (PC-3), renal cancer (UO-31 and RXF 393), and ovarian cancer (OVCAR-8) cell lines. Both compounds showed similar activity against breast cancer (T-47D and MDA-MB-231), colon cancer (HCC-2998 and HCT-116), renal cancer (UO-31, 786–0, and SN 12C), and ovarian cancer (OVCAR-3) cell lines.

Although structural similarity was observed between sidrin and betulinic acid derivatives, the presence of a free hydroxyl group on the C-3 of the latter was considered essential for their cytotoxic effect (Gauthier et al., 2006; Kommera et al., 2010; Mukherjee et al., 2006; Podolak et al., 2022; Shu et al., 2022). However, our findings indicated that this assumption was incorrect because the hydroxyl group in the same position on sidrin was found to form an epoxy ring with C-2, and the compound proved effective. In addition, recent studies have reported that the cytotoxic activity depends on the type of substitution on C-3 and C-28, not on the presence of free hydroxyl on C-3 or the free carboxylic group on C-28 (Kommera et al., 2010; Kozubek et al., 2021; Ling et al., 2022; Nistor et al., 2022). Moreover, the cytotoxic activity, but with decreased selectivity, was increased by the oxidation of this hydroxyl group. This activity might have been increased by introducing chemical groups, such as hydroxyl, at the C-28 position (Seca and Pinto, 2018).

Triterpenoid saponins exhibit different biological activities. Dammarane-type triterpenoid saponins (bacopaside II, bacopasaponin C, and bacoside A3, isolated from *Bacopa monniera* Wettst., Scrophulariaceae) exert mild-to-moderate cytotoxicity on non-cancerous kidney cell lines (Vero/LLC-PK1); however, they did not show any anticancer activity against five tumor cell lines (HT-29: colorectal epithelial carcinoma; SK-OV-3: ovary carcinoma; BT-549: breast ductal carcinoma; KB: oral epidermal carcinoma; and SK-MEL: malignant melanoma) (Pawar et al., 2007). On the other hand, the jujubogenin-type saponins are known to exhibit antidiabetic, hypnotic, antihyperglycemic, and immunological adjuvant effects (Matsuda et al., 1999). However, sidroside is the first jujubogenin glycoside to exhibit selective cytotoxicity against different cancer cells.

Interestingly, while sidrin and sidroside showed cytotoxicity in a dose-dependent manner for cell lines (HeLa, THP-1 monocyte, and bone marrow), neither of the compounds showed cytotoxic effects on normal bone marrow cells at the same or even higher

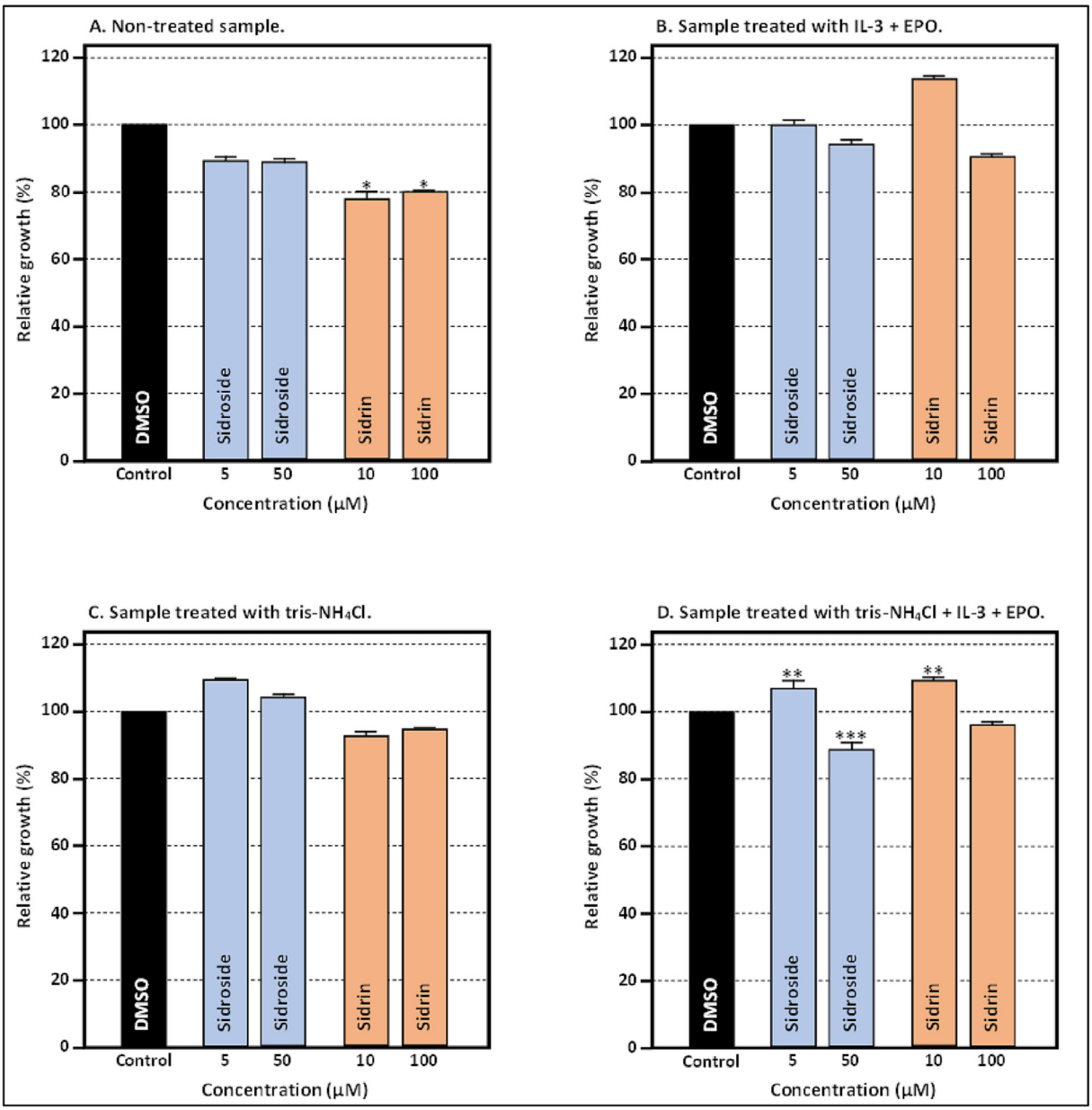

Fig. 6. The activity of sidroside and sidrin on the proliferation of cultured murine bone marrow cells against dimethyl sulfoxide (DMSO) as a control, expressed as the mean ± standard error of the mean (n = 3). **A.** Nothing was added to the sample; **B.** interleukin-3 (IL-3) and erythropoietin (EPO) were added; **C.** tris-ammonium chloride (tris-NH4Cl) was added; and **D.** IL-3, EPO, and tris-NH_4Cl were added. A significant difference is expressed as the following: (*) for $p < 0.05$, (**) for $p < 0.01$, and (***) for $p < 0.001$.

concentrations used for tumor cells. This finding suggests tumor-selective cytotoxicity of sidrin and sidroside, with a high selectivity index.

5. Conclusions

Here, the isolation, structure determination, and selective anti-tumor activity of two novel triterpenoids, sidrin and sidroside, from the leaves of *Z. spina-christi* have been described. Selective cytotoxicity of both compounds was observed against the HeLa, THP-1, and 60 other cancer cell lines from the NCI, whereas no significant effects were observed on the proliferation of normal bone marrow cells. Both compounds had comparatively moderate to high IC_{50} values against the cancer cells evaluated in this study and were found to have antiproliferative activity. The findings of this study suggest that sidrin and sidroside are promising compounds that may contribute to the development of anticancer drugs. Alternatively, they may be combined with existing antineoplastic medications to enhance their therapeutic benefits. However, further *in vitro* and in vivo mechanistic research is necessary to fully comprehend the potential value of sidrin and sidroside.

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Author contributions

Study conception and design: MAHM and TO; chemical analysis and manuscript drafting: MAHM; data analysis, visualization, and writing: HMJK.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2023.04.029.

References

Abdel Bar, F.M., Khanfar, M.A., Elnagar, A.Y., Liu, H., Zaghloul, A.M., Badria, F.A., Sylvester, P.W., Ahmad, K.F., Raisch, K.P., El Sayed, K.A., 2009. Rational design and semisynthesis of betulinic acid analogues as potent topoisomerase inhibitors. J Nat Prod 72, 1643–1650. https://doi.org/10.1021/NP900312U.

Abdel-galil, F.M., El-Jissry, M.A., 1991. Cyclopeptide alkaloids from Zizyphus spina-christi. Phytochemistry 30, 1348–1349. https://doi.org/10.1016/S0031-9422(00)95238-5.

Abdel-Zaher, A.O., Salim, S.Y., Assaf, M.H., Abdel-Hady, R.H., 2005. Antidiabetic activity and toxicity of Zizyphus spina-christi leaves. J Ethnopharmacol 101, 129–138. https://doi.org/10.1016/j.jep.2005.04.007.

Abdullah, Khan, M.A., Ahmad, W., Ahmad, M., Adhikari, A., Ibrar, M., Rehman, M. ur, Asif, M., 2022. Antioxidant, antinociceptive, anti-inflammatory, and hepatoprotective activities of pentacyclic triterpenes isolated from Ziziphus oxyphylla Edgew. Drug Chem Toxicol 45, 1796–1807. https://doi.org/10.1080/01480545.2021.1880427.

Abu-Rabia, A., 2015. Indigenous medicine among the Bedouin in the Middle East. Berghahn Books, New York, pp. 90–92. https://doi.org/10.3167/9781782386896.

Adam, I.A., Irshad, R., Atia-tul-Wahab, Omoboyowa, D.A., Choudhary, M.I., Wang, Y., 2022. Two new 5(14)-membered type cyclopeptide alkaloids from root bark of Ziziphus spina-christi (L.) Desf. Nat Prod Res 1–7. https://doi.org/10.1080/14786419.2022.2050227.

Adly, E., Hegazy, A.A., Kamal, M., Abu-Hussien, S.H., 2022. Midguts of Culex pipiens L. (Diptera: Culicidae) as a potential source of raw milk contamination with pathogens. Sci Rep 12, 13183. https://doi.org/10.1038/s41598-022-16992-9.

Adzu, B., Amos, S., Wambebe, C., Gamaniel, K., 2001. Antinociceptive activity of Zizyphus spina-christi root bark extract. Fitoterapia 72, 344–350. https://doi.org/10.1016/S0367-326X(00)00289-6.

Adzu, B., Amos, S., Amizan, M.B., Gamaniel, K., 2003. Evaluation of the antidiarrhoeal effects of Zizyphus spina-christi stem bark in rats. Acta Trop 87, 245–250. https://doi.org/10.1016/S0001-706X(03)00114-1.

Adzu, B., Haruna, A.K., 2010. Studies on the use of Zizyphus spina-christi against pain in rats and mice. Afr J Biotechnol 6, 1317–1324. https://doi.org/10.4314/ajb.v6i11.57480.

Al-Fatimi, M., 2019. Ethnobotanical survey of medicinal plants in central Abyan governorate. Yemen. J Ethnopharmacol 241. https://doi.org/10.1016/j.jep.2019.111973.

Alsayari, A., Wahab, S., 2021. Genus Ziziphus for the treatment of chronic inflammatory diseases. Saudi J Biol Sci 28, 6897–6914. https://doi.org/10.1016/j.sjbs.2021.07.076.

Czarnotta, E., Dianat, M., Korf, M., Granica, F., Merz, J., Maury, J., Baallal Jacobsen, S. A., Förster, J., Ebert, B.E., Blank, L.M., 2017. Fermentation and purification strategies for the production of betulinic acid and its lupane-type precursors in Saccharomyces cerevisiae. Biotechnol Bioeng 114, 2528–2538. https://doi.org/10.1002/BIT.26377.

Dhanalekshmi, U.M., Alam, T., Khan, S.A., 2022. The folkloric uses and economic importance of some selected edible medicinal plants native to Oman: a brief overview, in: Masoodi, M.H., Rehman, M.U. (Eds.), Edible Plants in Health and Diseases : Volume 1 : Cultural, Practical and Economic Value. Springer Nature Singapore, Singapore, pp. 1–29. https://doi.org/10.1007/978-981-16-4880-9_1.

Dkhil, M.A., Kassab, R.B., Al-Quraishy, S., Abdel-Daim, M.M., Zrieq, R., Abdel Moneim, A.E., 2018. Ziziphus spina-christi (L.) leaf extract alleviates myocardial and renal dysfunction associated with sepsis in mice. Biomedicine & Pharmacotherapy 102, 64–75. https://doi.org/10.1016/j.biopha.2018.03.032.

Duke, J.A., Ayensu, E.S., 1985. Medicinal Plants of China. Reference Publications, Medicinal plants of the world.

El Kutry, M.S., Sopeah, H.R.A., 2020. Impact of intake a sidr (Zizyphus spina-christi L.) extract on enterobiasis vermicularis infection for children. Biomedical and Pharmacology Journal 13, 359–366. https://doi.org/10.13005/BPJ/1896.

El Maaiden, E., El Kharrassi, Y., Moustaid, K., Essamadi, A.K., Nasser, B., 2019. Comparative study of phytochemical profile between Ziziphus spina christi and Ziziphus lotus from Morocco. Journal of Food Measurement and Characterization 13, 121–130. https://doi.org/10.1007/s11694-018-9925-y.

Elghaffar, R.Y.A., Amin, B.H., Hashem, A.H., Sehim, A.E., 2022. Promising endophytic Alternaria alternata from leaves of Ziziphus spina-christi: phytochemical analyses, antimicrobial and antioxidant activities. Appl Biochem Biotechnol 194, 3984–4001. https://doi.org/10.1007/s12010-022-03959-9.

El-Shahir, A.A., El-Wakil, D.A., Latef, A.A.H.A., Youssef, N.H., 2022. Bioactive compounds and antifungal activity of leaves and fruits methanolic extracts of Ziziphus spina-christi L. Plants (Basel) 11. https://doi.org/10.3390/PLANTS11060746.

Gauthier, C., Legault, J., Lebrun, M., Dufour, P., Pichette, A., 2006. Glycosidation of lupane-type triterpenoids as potent in vitro cytotoxic agents. Bioorg Med Chem 14, 6713–6725. https://doi.org/10.1016/J.BMC.2006.05.075.

Ghazanfar, S.A., 1994. Handbook of Arabian medicinal plants. CRC Press, London, p. 182. https://doi.org/10.1007/BF02863093.

Glombitza, K.W., Mahran, G.H., Mirhom, Y.W., Michel, K.G., Motawi, T.K., 1994. Hypoglycemic and antihyperglycemic effects of Zizyphus spina-christi in rats. Planta Med 60, 244–247. https://doi.org/10.1055/S-2006-959468.

Hamedi, A., Bayat, M., Asemani, Y., Amirghofran, Z., 2022. A review of potential anticancer properties of some selected medicinal plants grown in Iran. J Herb Med 33. https://doi.org/10.1016/j.hermed.2022.100557.

Hordyjewska, A., Ostapiuk, A., Horecka, A., Kurzepa, J., 2019. Betulin and betulinic acid: triterpenoids derivatives with a powerful biological potential. Phytochemistry Reviews 18, 929–951. https://doi.org/10.1007/s11101-019-09623-1.

Hussein, A.S., 2019. Ziziphus spina-christi: Analysis of Bioactivities and Chemical Composition. In: Mariod, A.A. (Ed.), Wild Fruits: Composition, Nutritional Value and Products. Springer International Publishing, Cham, pp. 175–197. https://doi.org/10.1007/978-3-030-31885-7_15.

Hussein, H.M., El-Sayed, E.M., Said, A.A., 2006. Antihyperglycemic, antihyperlipidemic and antioxidant effects of Zizyphus spina christi and Zizyphus jujuba in alloxan diabetic rats. International Journal of Pharmacology 2, 563–570. https://doi.org/10.3923/IJP.2006.563.570.

Ibn Sina [Avicenna], 1025. The Canon of Medicine. Medical Press, Rome, p. 217.

Ildstad, S.T., Sachs, D.H., 1984. Reconstitution with syngeneic plus allogeneic or xenogeneic bone marrow leads to specific acceptance of allografts or xenografts. Nature 307, 168–170. https://doi.org/10.1038/307168A0.

Kommera, H., Kaluđerović, G.N., Dittrich, S., Kalbitz, J., Dräger, B., Mueller, T., Paschke, R., 2010. Carbamate derivatives of betulinic acid and betulin with selective cytotoxic activity. Bioorg Med Chem Lett 20, 3409–3412. https://doi.org/10.1016/J.BMCL.2010.04.004.

Kozubek, M., Höhlich, L., Hoenke, S., Deigner, H.-P., Al-Harrasi, A., Csuk, R., 2021. Apoptotic activity of substituted 3-O-acetyl-betulinic acid benzylamides. European Journal of Medicinal Chemistry Reports 3. https://doi.org/10.1016/j.ejmcr.2021.100016.

Le Crouéour, G., Thépenier, P., Richard, B., Petermann, C., Ghédira, K., Zèches-Hanrot, M., 2002. Lotusine G: a new cyclopeptide alkaloid from Zizyphus lotus. Fitoterapia 73, 63–68. https://doi.org/10.1016/S0367-326X(01)00363-X.

Ling, T., Boyd, L., Rivas, F., 2022. Triterpenoids as reactive oxygen species modulators of cell fate. Chem Res Toxicol 35, 569–584. https://doi.org/10.1021/acs.chemrestox.1c00428.

Marmouzi, I., Kharbach, M., El Jemli, M., Bouyahya, A., Cherrah, Y., Bouklouze, A., Vander Heyden, Y., Faouzi, M.E.A., 2019. Antidiabetic, dermatoprotective, antioxidant and chemical functionalities in Zizyphus lotus leaves and fruits. Ind Crops Prod 132, 134–139. https://doi.org/10.1016/j.indcrop.2019.02.007.

Matsuda, H., Murakami, T., Ikebata, A., Yamahara, J., Yoshikawa, M., 1999. Bioactive saponins and glycosides. XIV. Structure elucidation and immunological adjuvant activity of novel protojujubogenin type triterpene bisdesmosides, protojujubosides A, B, and B1, from the seeds of Zizyphus jujuba var. spinosa (Zizyphi Spinosi Semen). Chem Pharm Bull (Tokyo) 47, 1744–1748. https://doi.org/10.1248/CPB.47.1744.

Mostafa, M., Takano, F., Ohta, T., 2010. Unique phenyl ether type biflavonoids, zizyflavosides A and B with biomimetic synthesis for zizyflavoside A. Heterocycles 81, 2497–2509. https://doi.org/10.3987/COM-10-12002.

Mukherjee, R., Kumar, V., Srivastava, S.K., Agarwal, S.K., Burman, A.C., 2006. Betulinic acid derivatives as anticancer agents: structure activity relationship. Anticancer Agents Med Chem 6, 271–279. https://doi.org/10.2174/187152006776930846.

Neamah, B.A.H., Alquraishy, M.K., Almousawi, A.H., Chessab, R.M., Shaker, Z.B., Abed, H.A., 2022. Antibiofilm impact of ziziphus spina christi on methicillin resistance

of Staphylococcus warneri. Int J Health Sci (Qassim) 6, 5492–5499. https://doi.org/10.53730/ijhs.v6nS1.5651.
Nesseem, D.I., Michel, C.G., Sleem, A.A., El-Alfy, T.S., 2009. Formulation and evaluation of antihyperglycemic leaf extracts of Zizyphus spina-christi (L.) Willd. Pharmazie 64, 104–109. https://doi.org/10.1691/ph.2008.8614.
Niksic, H., Becic, F., Koric, E., Gusic, I., Omeragic, E., Muratovic, S., Miladinovic, B., Duric, K., 2021. Cytotoxicity screening of Thymus vulgaris L. essential oil in brine shrimp nauplii and cancer cell lines. Sci Rep 11, 13178. https://doi.org/10.1038/s41598-021-92679-x.
Nistor, G., Trandafirescu, C., Prodea, A., Milan, A., Cristea, A., Ghiulai, R., Racoviceanu, R., Mioc, A., Mioc, M., Ivan, V., Șoica, C., 2022. Semisynthetic derivatives of pentacyclic triterpenes bearing heterocyclic moieties with therapeutic potential. Molecules 27, 6552. https://doi.org/10.3390/molecules27196552.
Pawar, R.S., Khan, S.I., Khan, I.A., 2007. Glycosides of 20-deoxy derivatives of jujubogenin and pseudojujubogenin from Bacopa monniera. Planta Med 73, 380–383. https://doi.org/10.1055/S-2007-967163.
Pawlowska, A.M., Camangi, F., Bader, A., Braca, A., 2009. Flavonoids of Zizyphus jujuba L. and Zizyphus spina-christi (L.) Willd (Rhamnaceae) fruits. Food Chem 112, 858–862. https://doi.org/10.1016/j.foodchem.2008.06.053.
Podolak, I., Grabowska, K., Sobolewska, D., Wróbel-Biedrawa, D., Makowska-Wąs, J., Galanty, A., 2022. Saponins as cytotoxic agents: an update (2010–2021). Part II—Triterpene saponins. Phytochemistry Reviews In Press. https://doi.org/10.1007/s11101-022-09830-3.
Qiao, W., Zhou, Z., Liang, Q., Mosongo, I., Li, C., Zhang, Y., 2019. Improving lupeol production in yeast by recruiting pathway genes from different organisms. Sci Rep 9, 2992. https://doi.org/10.1038/s41598-019-39497-4.
Rubinstein, L.V., Shoemaker, R.H., Paull, K.D., Simon, R.M., Tosini, S., Skehan, P., Scudiero, D.A., Monks, A., Boyd, M.R., 1990. Comparison of in vitro anticancer-drug-screening data generated with a tetrazolium assay versus a protein assay against a diverse panel of human tumor cell lines. J Natl Cancer Inst 82, 1113–1117. https://doi.org/10.1093/JNCI/82.13.1113.
Sakna, S.T., Maghraby, Y.R., Abdelfattah, M.S., Farag, M.A., 2022. Phytochemical diversity and pharmacological effects of triterpenes from genus Ziziphus: a comprehensive review. Phytochemistry Reviews In Press. https://doi.org/10.1007/s11101-022-09835-y.
Santana Gomes, J.N., Santos, M.B., de Medeiros e Silva, Y.M.S., Albino, S.L., de Moura, R.O., 2022. Topoisomerase enzyme inhibitors as potential drugs against cancer: what makes them selective or dual? – A review. Curr Pharm Des 28. https://doi.org/10.2174/1381612828666220728095619.
Seca, A.M.L., Pinto, D.C.G.A., 2018. Plant secondary metabolites as anticancer agents: successes in clinical trials and therapeutic application. Int J Mol Sci 19, 263. https://doi.org/10.3390/IJMS19010263.
Seri, C.S., Okpekon, T.A., Yao-Kouassi, P.A., Magid, A.A., Sayagh, C., Voutquenne-Nazabadioko, L., 2020. Saponins and flavonoid glycosides from the leaves of Ziziphus mauritiana Lam. native of a forest area of Ivory Coast. Phytochem Lett 37, 5–9. https://doi.org/10.1016/J.PHYTOL.2020.03.001.
Shakiba, R., Nilforoushzadeh, M.A., Dabaghian, F., Zangii, B., Ghobadi, A., Shirbeigi, L., Aliasl, J., Shakiba, M., Ghods, R., 2019. Effect of Cedar (Ziziphus spina-christi) topical solution in mild to moderate acne vulgaris: a randomized clinical study. Journal of Dermatological Treatment 32, 1–6. https://doi.org/10.1080/09546634.2019.1692125.
Shen, C.-Y., Wan, L., Zhu, J.-J., Jiang, J.-G., 2020. Targets and underlying mechanisms related to the sedative and hypnotic activities of saponin extracts from semen Ziziphus jujube. Food Funct 11, 3895–3903. https://doi.org/10.1039/D0FO00098A.
Shergis, J.L., Ni, X., Sarris, J., Zhang, A.L., Guo, X., Xue, C.C., Lu, C., Hugel, H., 2017. Ziziphus spinosa seeds for insomnia: a review of chemistry and psychopharmacology. Phytomedicine 34, 38–43. https://doi.org/10.1016/j.phymed.2017.07.004.
Shoemaker, R.H., 2006. The NCI60 human tumour cell line anticancer drug screen. Nat Rev Cancer 6, 813–823. https://doi.org/10.1038/NRC1951.
Shu, Y., Li, F., Han, Y., Wang, P., Gao, F., Yan, M., Liang, M., Ma, Q., Zhang, Y., Ding, X., Lei, H., 2022. Design, synthesis and cytotoxic evaluation of novel betulonic acid-diazine derivatives as potential antitumor agents. Front Chem 10. https://doi.org/10.3389/FCHEM.2022.969770.
Siddiqui, A.J., Jahan, S., Singh, R., Saxena, J., Ashraf, S.A., Khan, A., Choudhary, R.K., Balakrishnan, S., Badraoui, R., Bardakci, F., Adnan, M., 2022. Plants in anticancer drug discovery: from molecular mechanism to chemoprevention. Biomed Res Int 5425485.
Skehan, P., Storeng, R., Scudiero, D., Monks, A., Mcmahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S., Boyd, M.R., 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst 82, 1107–1112. https://doi.org/10.1093/JNCI/82.13.1107.
Takım, K., 2021. Bioactive component analysis and investigation of antidiabetic effect of Jerusalem thorn (Paliurus spina-christi) fruits in diabetic rats induced by streptozotocin. J Ethnopharmacol 264. https://doi.org/10.1016/J.JEP.2020.113263.
Talukdar, A., Kundu, B., Sarkar, D., Goon, S., Mondal, M.A., 2022. Topoisomerase I inhibitors: challenges, progress and the road ahead. Eur J Med Chem 236. https://doi.org/10.1016/j.ejmech.2022.114304.
Tanira, M.O.M., Ageel, A.M., Tariq, M., Mohsin, A., Shah, A.H., 1988. Evaluation of some Pharmacological, Microbiological and Physical Properties of Zizyphus spina-christi. Int J Crude Drug Res 26, 56–60. https://doi.org/10.3109/13880208809053889.
Tuenter, E., Foubert, K., Staerk, D., Apers, S., Pieters, L., 2017. Isolation and structure elucidation of cyclopeptide alkaloids from Ziziphus nummularia and Ziziphus spina-christi by HPLC-DAD-MS and HPLC-PDA-(HRMS)-SPE-NMR. Phytochemistry 138, 163–169. https://doi.org/10.1016/J.PHYTOCHEM.2017.02.029.
Valencia-Chan, L.S., Moreno-Lorenzana, D., Ceballos-Cruz, J.J., Peraza-Sánchez, S.R., Chávez-González, A., Moo-Puc, R.E., 2022. Apoptotic and cell cycle effects of triterpenes isolated from Phoradendron wattii on leukemia cell lines. Molecules 27. https://doi.org/10.3390/molecules27175616.
Wang, T., Guo, S., Ren, X., Du, J., Bai, L., Cui, X., Ho, C.-T., Bai, N., 2022a. Simultaneous quantification of 18 bioactive constituents in Ziziphus jujuba fruits by HPLC coupled with a chemometric method. Food Science and Human Wellness 11, 771–780. https://doi.org/10.1016/j.fshw.2022.03.003.
Wang, Z., Han, L., Zhou, Y., Cai, J., Sun, S., Ma, J., Wang, W., Li, X., Ma, L., 2022b. The combination of a 3D-Printed porous Ti–6Al–4V alloy scaffold and stem cell sheet technology for the construction of biomimetic engineered bone at an ectopic site. Mater Today Bio 16. https://doi.org/10.1016/j.mtbio.2022.100433.
Wróblewska-Łuczka, P., Cabaj, J., Bąk, W., Bargieł, J., Grabarska, A., Góralczyk, A., Łuszczki, J.J., 2022. Additive interactions between betulinic acid and two taxanes in in vitro tests against four human malignant melanoma cell lines. Int J Mol Sci 23. https://doi.org/10.3390/ijms23179641.
Xu, D., Xiao, J., Jiang, D., Liu, Y., Gou, Z., Li, J., Shi, M., Wang, X., Guo, Y., Ma, L., Yin, H., Guo, L., Zhu, C., Zhang, Y., Guo, H., 2022. Inhibitory effects of a water-soluble jujube polysaccharide against biofilm-forming oral pathogenic bacteria. Int J Biol Macromol 208, 1046–1062. https://doi.org/10.1016/j.ijbiomac.2022.03.196.
Zuco, V., Supino, R., Righetti, S.C., Cleris, L., Marchesi, E., Gambacorti-Passerini, C., Formelli, F., 2002. Selective cytotoxicity of betulinic acid on tumor cell lines, but not on normal cells. Cancer Lett 175, 17–25. https://doi.org/10.1016/S0304-3835(01)00718-2.