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Full Length Research Paper

Cytotoxic activity of *Agave lechuguilla* Torr

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The cytotoxic activity of extract and isolated saponin from leaves of *Agave lechuguilla* was investigated. Ethanol extract from leaves of *A. lechuguilla* exhibited cytotoxic activity against HeLa cells *in vitro* (50% inhibitory concentration (IC_{50}) = 89 μg/ml). Bioassay-guided fractionation of this extract had led to the isolation of 5-β steroidal saponin with an IC_{50} value against HeLa cells of 78 μg/ml. Neither the extract nor saponin showed significant cytotoxicity against MCF-7 cells (IC_{50} > 150 μg/ml). The extract and isolated saponins were tested for their cytotoxicity against Vero cells, where they showed IC_{50} values of 126, 171.6 and 172.2 μg/ml, respectively.

Key words: *Agave lechuguilla*, cytotoxicity, plant extract, saponin.

INTRODUCTION

Tumor resistance to standard chemotherapy drugs, either initially or after repeated cycles, remains an obstacle for successful treatment of cancer patients (Lobert et al., 2011). The incidence and mortality of cancer have increased, and new agents with antitumor activity are needed. Plants are used in pharmaceutical treatments. *Catharanthus roseus* plant produces important alkaloids, including antineoplastic medicines such as vinblastine and vincristine (Kimura and Okuda, 2000).

Plants belonging to the family of Agavaceae, which includes more than 300 species, occur natively in the arid and tropical regions of the Western Hemisphere, particularly in Mexico and Central America. Agavaceae is known to be a rich source of steroidal saponins, fiber, and steroidal sapogenins (Yokosuka et al., 2000).

The agave plant *Agave lechuguilla* Torr (family Agavaceae) is found in the Chihuahuan, Coahuilan, and Sonoran deserts in Mexico, where it grows always on limestone. The flowers are a source of nutrients forinsects, bats, and some birds. The leaves are long, tough, and rigid, with very sharp, hard points that can penetrate clothing and skin easily. Native Americans use the fibers from the leaves (commonly called *ixtle*) to make ropes and mats (Hernández et al., 2005). A previous study of *A. americana* reported a new saponin, along with three known saponins (Yokosuka et al., 2000). The isolated saponin, hecogenin tetraglycoside, showed cytotoxic activity against HL-60 human promyelocytic leukemia cells (Barret, 2001; Roja and Rao, 2000; Yokosuka et al., 2000). The aim of this study was to evaluate the cytotoxic activity of crude extracts and isolated saponins of *A. lechuguilla* against cancer cell lines from cervical (HeLa) and mammary gland (MCF-7) tissues and from non cancer kidney (Vero) cells.

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Abbreviations: ATCC, American type culture collection; **IC_{50},** concentration required to inhibit 50% of the initial cell viability of controls; **LD_{50},** 50% lethal dose; **SI,** selective index.

MATERIALS AND METHODS

Plant source

Specimens of *A. lechuguilla* were collected from Monterrey, Nuevo Leon, in Mexico. The botanical identification and authentication were confirmed at the Department of Botany of the Autonomous University of Nuevo Leon, where a voucher specimen is kept at the

Table 1. Cytotoxicity (μg/ml) of ethanol extract and saponins isolated from *A. Lechuguilla* for MCF-7, HeLa, and Vero cells.

IC_{50} (μg/ml)[a]	MCF-7	HeLa	Vero	SI[b]
Ethanol extract	>150	89	126	1.42
Crude saponin	>150	95	171.6	1.81
Crystallized saponin	>150	78	172.2	2.21

[a]Each value represents the mean of three independent experiments. [b]Selectivity index (SI) of ethanol extract and isolated saponins compared with Vero cells.

herbarium (Voucher no. 024190).

Preparation of ethanol extract

A. lechuguilla leaves were air-dried at room temperature and cut into pieces of about 1 cm^2. Ethanol extraction was performed on a shaker at room temperature for 7 days. The filtrate obtained was evaporated to dryness using a rotary evaporator.

Brine shrimp lethality test

The brine shrimp lethality assay was used as an initial screening assay of cytotoxic activity (McLaughlin et al., 1991; Meyer et al., 1982). Brine shrimp (*Artemia salina*) eggs were first eclosionated hatched for 48 h in artificial seawater. A stock ethanol extract was prepared by diluting the original ethanol extract of *A. lechuguilla* to a concentration of 40 mg/ml with artificial seawater. Five concentrations of ethanol extract (0, 100, 300, 500, and 1,000 μg/ml) were tested. The positive controls contained 5% $K_2Cr_2O_7$. The test was conducted using 7–10 hatched brine shrimp per 100 μl of salt water in 96-well microplates. Aliquots of the ethanol extract (100 μl) were added to the wells containing the brine shrimp, and the plates were incubated at room temperature for 24 h. The numbers of dead and live brine shrimp were recorded and used to estimate viability and the 50% lethal dose (LD_{50}). The results are expressed as the mean of three independent experiments conducted in triplicate.

Cytotoxicity assay

An extract of *A. lechuguilla* showing an $LD_{50} \leq 100$ μg/ml was selected for the measurement of cytotoxic activity against HeLa cells (ATCC number HTB-22), MCF-7 cells (ATCC number CCL-2), and Vero cells (ATCC number CCL-81). Cells were cultured in 25 ml flasks. The HeLa cell line was grown in Dulbecco's modified essential medium (DMEM), containing 2 mM L-glutamine, penicillin (100 IU/ml), and streptomycin (100 mg/ml) supplemented with 10% fetal bovine serum (Sigma-Aldrich Chemical, Co., St Louis, MO, USA). The MCF-7 cell line was maintained in RPMI 1640 medium (Gibco-Invitrogen, cat. 1875, Grand Island, NY, USA) containing penicillin (100 IU/ml) and streptomycin (100 mg/ml) supplemented with 10% fetal bovine serum. The Vero cell line was maintained in M-199 medium (Gibco, cat. 11150-059) with penicillin (100 IU/ml) and streptomycin (100 mg/ml) supplemented with 4% fetal bovine serum. Cells were grown in a humidified atmosphere of 5% CO_2 and 95% air at 37°C.

Cells were subcultured according to standard trypsinization procedures. Cell lines (3×10^5 cells) in 100 μl aliquots were inoculated into each well of a set of microplates and incubated at 37°C for 24 h, after which 100 μl aliquots of the extracts were added to the wells at different dilutions (10, 100, or 1000 μg/ml). Cells were grown at 37°C for 48 h, and the number of viable cells per well was measured using the WST-1 viability assay (Ishiyama et al., 1993), and the absorbance at 545 nm wavelength was plotted. The absorbance at 545 nm was linearly related to the proportion of viable cells. The cytotoxicity of test compounds is expressed as the IC_{50}, defined as the concentration of the test compounds required to inhibit 50% of the initial cell viability of controls. The standard test used 1.0 μM paclitaxel (Sigma-Aldrich Chemical, Co., St Louis, MO, USA). The results are expressed as the mean of three independent experiments conducted in triplicate.

Phytochemical analysis

The functional groups and secondary metabolites were identified using specific chemical reactions for alkaloids, coumarins, saponins, sesquiterpene lactone, sterols, and triterpenes (Cannell, 1998).

Isolation of saponins

Saponins of *A. lechuguilla* were isolated using 50 g of the plant and 150 ml of 80% ethanol in a Soxhlet extractor (Hernández et al., 2005). The mix was distilled four times and then evaporated in an oven at 40°C. Two grams of ethanol extract was obtained and placed in a conical tube, 5 ml of 50% ethanol was added followed by 5 ml of benzene, and the tube was vortexed for 15 min and centrifuged at 200 g for 15 min. Saponin was obtained from the interface formed. To obtain crystals, the extracts were placed in a 50 ml flask; 0.60 g of crude saponin was obtained with 25 ml of 50% ethanol and 3 ml of a solution of KOH in 1 mol/L ethanol. The extract was refluxed for 2 h, transferred to a vial of known weight, and evaporated to form crystallized saponin. The saponin produced was characterized using nuclear magnetic resonance spectroscopy of protons and carbon 13 (^{1}HRMN and ^{13}CRMN, respectively) using a Bruker 400 MHz DPX-400 spectrometer (Germany).

RESULTS AND DISCUSSION

The ethanol extract of *A. lechuguilla* showed significant activity at ≤ 100 μg/ml in the brine shrimp assay with an LD_{50} value of 46.3. In the WST-1 viability assay, crystallized saponin and the ethanol extract showed greater cytotoxic activity against HeLa cells than did the other extracts. Saponin was also less cytotoxic to normal Vero cells (Table 1). Some plants contain toxic compounds with anticancer activity (Bifulco and DiMarzo, 2003; Zhou et al., 2004). The brine shrimp lethality test with *A. salina* is used widely to select crude extracts with medical potential this lethality test correlates well with cytotoxicity (Lellau and Liebezeit, 2003).

The ethanol extract was selected for further cytotoxicity bioassays on tumor cells because it showed activity against *A. salina*. The WST-1 assay in the two tumor cell lines showed that the ethanol extract of *A. lechuguilla* exerted greater cytotoxicity against the HeLa cell line, with an IC_{50} of 89 μg/ml and less cytotoxicity against MCF-7 cells (IC_{50} >150 μg/ml). Crude and crystallized saponin extracted from *A. lechuguilla* had IC_{50} values of 95 and 78 μg/ml against HeLa cells, respectively but the saponin extract did not exert significant cytotoxicity against MCF-7 cells (IC_{50} > 150 μg/ml) (Table 1). The MCF-7 breast carcinoma cell line is relatively insensitive to many chemotherapeutic agents and represents a widely used model for the analysis of chemoresistance in breast cancer (Simstein et al., 2003).

The ethanol extract of *A. lechuguilla* produced an IC_{50} of 126 μg/ml against Vero cells. The selective index (SI) for the ethanol extract on HeLa cells compared with normal Vero cells was 1.4, whereas crude and crystallized saponin extracts of *A. lechuguilla* produced IC_{50} values of 171.6 and 172.2 μg/ml against Vero cells, respectively. The SI values for crude and crystallized saponin extract in HeLa cells compared with normal Vero cells were 1.81 and 2.21 (Table 1). Saponins from *A. americana* had an IC_{50} of 4.3 μg/ml against the human promyelocytic leukemia cell line HL-60 (Yokosuka et al., 2000). One saponin extracted from *A. fourcroydes* identified as chlorogenin had an IC_{50} of 7.5 to 10 μg/ml against HeLa cells (Ohtsuki et al., 2004). In the Liliaceae family, saponins showed an IC_{50} of approximately 5 mmol/L against mouse lymphocytic leukemia L1210 cells (Candra et al., 2002; Ohtsuki et al., 2004).

The ^{1}HRMN and ^{13}CRMN spectra from the crystallized saponin extract gave the following results for spiroacetal molecules: the ^{1}HRMN system produced δ values of 4.62 (C-16) and 3.51 (C-26), and the ^{13}CRMN analysis gave δ values of 81.2 (C-16), 114.7 (C-22), 36.8 (C-23), and 74.1 (C-26). These values have been reported for this type of structure (Candra et al., 2002). Similar ^{13}CRMN δ signals of 81.2 (C-16), 114.7 (C-22), 36.8 (C-23), and 74.1 (C-26) have been reported for other saponins (Madrigal and Cuevas, 1992; Sautour et al., 2005). These values are consistent with *cis* fusion of the A and B rings, indicating that our compound is a 5-β steroidal saponin.

Conclusion

This work contributes to the scientific study of northern Mexican plants with medical potential. To continue this study, we must isolate other compounds from *A. lechuguilla* and measure their cytotoxic activity levels against other tumor cell lines.

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REFERENCES

Barret BD (2001). Medicinal plants. J. Herbs Spices Med. Plants 8:1-35.

Bifulco M, DiMarzo V (2003). The endocannabinoid system as a target for the development of new drugs for cancer therapy. Recent Prog. Med. 94:194-198.

Candra E, Matsunaga K, Fujiwara H, Mimaki Y, Kuroda M, Sashida Y, Ohizumi Y (2002). Potent apoptotic effects of saponins from Liliaceae plants in L1210 cells. J. Pharm. Pharmacol. 2:257-262.

Cannell RJP (1998). Editor. Methods in Biotechnology Natural Products Isolation Vol. 4. Humana Press, Totowa, New Jersey, USA. pp. 112-137.

Hernández RS, Lugo EC, Díaz L, Villanueva S (2005). Indirect extraction and quantification of saponins of *Agave lechuguilla* Torr. Revista Digital Cientifica e-Gnosis. Año/vol. 3. Universidad de Guadalajara. Mexico. ISSN. Online February 2007: www.e-gnosis.udg.mx. [In Spanish].

Ishiyama M, Shiga M, Sasamoto K, Mizoguchi M, He P (1993). A new sulfonated tetrazolium salt that produces a highly water-soluble formazan dye. Chem. Pharm. Bull. 41:1118-1122.

Kimura Y, Okuda H (2000). Effects of naturally occurring stilbene glucosides from medicinal plants and wine, on tumor growth and lung metastasis in Lewis lung carcinoma-bearing mice. J. Pharm. Pharmacol. 52:1287-1295.

Lellau TF, Liebezeit G (2003). Cytotoxic and antitumor activities of ethanolic extracts of Salt Marsh plants from the lower Saxonian Wadden Sea, Southern North Sea. Pharm. Biol. 41:293-300.

Lobert S, Jefferson B, Morris K (2011). Regulation of β-tubulin isotypes by micro-RNA 100 in MCF7 breast cancer cells. Cytoskeleton 68:355-362.

Madrigal PD, Cuevas AC (1992). Identification of steroids in *Agave angustifolia.* Rev. Latinoam. Quim. 23:28-30.

McLaughlin JL, Chang CJ, Smith DL (1991). “Bench-top” bioassays for the discovery of bioactive natural products: an update. In: Rahman A, Editor, Studies in Natural Product Chemistry, Vol. 9. Elsevier, Amsterdam pp. 383-409.

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, MacLaughlin JL (1982). Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 45:31-34.

Ohtsuki T, Koyano T, Kowithayakorn T, Sakai S, Kawahara N, Goda Y, Yamaguchi N, Ishibashi M (2004). New chlorogenin hexasaccharide isolated from *Agave fourcroydes* with cytotoxic and cell cycle inhibitory activities. Bioorg. Med. Chem. 15:3841-3845.

Roja G, Rao PS (2000). Anticancer compounds from tissue cultures of medicinal plants. J. Herbs Spices Med. Plants 7:71-101.

Sautour M, Tomofumi M, Lacaille-Dubois M (2005). Steroidal saponins from *Smilax medica* and their antifungal activity. J. Nat. Prod. 68:1489-1493.

Simstein R, Burow M, Parker A, Weldon C, Beckman B (2003). Apoptosis, chemoresistance, and breast cancer: insights from the MCF-7 cell model system. Exp. Biol. Med. 228:995-1003.

Yokosuka A, Mimaki Y, Kuroda M, Sashida Y (2000). A new steroidal saponin from the leaves of *Agave americana.* Planta Med. 66:393-396.

Zhou H, Hamazaki A, Fontana JD, Takahashi H, Esumi T, Wandscheer CB, Tsujimoto H, Fukuyama Y (2004). New ring C-seco limonoids from Brazilian *Melia azedarach* and their cytotoxic activity. J. Nat. Prod. 67:1544-1547.