Pharmacogn J. 2023; 15(1): 119-127
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Anticancer and Neuroprotective Effects of the Triterpene Glycosides From Sea Cucumber *Holothuria imitans*

Marilú Roxana Soto-Vásquez[1,*], Paul Alan Arkin Alvarado-García[2], Demetrio Rafael Jara-Aguilar[1], Elda Maritza Rodrigo-Villanueva[1], José Gilberto Gavidia-Valencia[1], Iris Melina Alfaro-Beltrán[3], Bertha Mirella Alfaro-Ttito[4]

ABSTRACT

Introduction: Sea cucumbers has gained notoriety because possess a wide range of biological and pharmacological activities. In this sense, the aim of this work was to evaluate the anticancer and neuroprotective effects of the triterpene glycosides from sea cucumber *Holothuria imitans*. **Methods:** Triterpene glycosides were separated and purified by Reversed-phase high-performance liquid chromatography (RP-HPLC). Their structures were deduced by spectral analysis and chemical evidence. Cytotoxic activity was evaluated using normal African green monkey kidney epithelial cell line (VERO) and three cancer cell lines: cancer gastric (MKN-28), breast adenocarcinoma (MCF-7) and lung carcinoma (A-549). Besides, the neuroprotective effect was studied using the Cath.a-differentiated (CAD) cell line and human glial (Oligodendrocytic) hybrid cell line (MO3.13). **Results**: Two triterpene glycosides (Fuscocineroside C and Scabraside D) were isolated, which showed low cytotoxic activity against VERO cell line, and high cytotoxic activity against lines MKN-28, MCF-7 and A-549 cells, with IC_{50} between the ranges of 0.92 µmol/L to 2.61 µmol/L. The isolated triterpene glycosides showed the ability to regain mitochondrial viability in CAD and MO3.13 cells treated with neurotoxin (C2-ceramide) with statistically significant results ($p<0.05$). **Conclusion:** The triterpene glycosides Fuscocineroside C and Scabraside D isolated from sea cucumber *Holothuria imitans* show anticancer and neuroprotective potential and may be considered promising active principles for anticancer and neuroprotective drugs.
Key words: *Holothuria imitans*, Triterpene glycosides, Anticancer, Neuroprotective.

Marilú Roxana Soto-Vásquez[1,*], Paul Alan Arkin Alvarado-García[2], Demetrio Rafael Jara-Aguilar[1], Elda Maritza Rodrigo-Villanueva[1], José Gilberto Gavidia-Valencia[1], Iris Melina Alfaro-Beltrán[3], Bertha Mirella Alfaro-Ttito[4]

[1]*Facultad de Farmacia y Bioquímica. Universidad Nacional de Trujillo, PERÚ.*

[2]*Escuela de Medicina. Universidad César Vallejo, Trujillo, PERÚ.*

[3]*Instituto Pablo Casals, Trujillo, PERÚ.*

[4]*Escuela de Posgrado de la Universidad Nacional de Trujillo, PERÚ.*

Correspondence

Marilú Roxana Soto-Vásquez

Facultad de Farmacia y Bioquímica. Universidad Nacional de Trujillo, PERÚ.

E-mail: msoto@unitru.edu.pe

History

- Submission Date: 02-11-2022;
- Review completed: 04-01-2023;
- Accepted Date: 06-01-2023.

DOI : 10.5530/pj.2023.15.16

Article Available online

http://www.phcogj.com/v15/i6

INTRODUCTION

Cancer is the first cause of death worldwide, responsible for an average 10 million deaths, and considered a public health problem whose morbidity is increasing as in developing and developed countries.[1] Even though, cancer treatments have shown a significant progress lately, the side effects of anticancer drugs still produce prejudicial responses that counteract the benefits of treatment.[2]

Moreover, memory and cognitive disorders such as Alzheimer disease and Parkinson's disease, are showing an upward trend; in fact, mortality increased significantly due to these ailments.[3] However, psychotropic drugs prescribed to patients with these conditions may cause severe adverse events and unwanted side effects.[4]

Natural products (NPs) have played a significant role in drug discovery due to their enormous scaffold diversity and structural complexity which are keys to their therapeutic potential.[5] Despite of terrestrial plants and bacteria have traditionally been considered as sources of NPs, marine organisms have also produced novel structural compound, known as marine natural products (MNPs).[6]

In this sense, MNPs have shown an immense potential in the treatment of human illnesses; in fact, evidence demonstrates the antiviral, antitumoral and anti-inflammatory effects of these compounds, in addition to improve the immune function.[7,8] In fact, There are various marine organisms which are sources of MNPs, nevertheless the phylum Echinodermata is the one of the most significant sources; and inside this, the sea cucumbers from the class Holothuroidea, are one of the major sources of glycosylated compounds.[9]

Generally, Sea cucumber glycosides have attracted special interest due to their cancer preventive and immunomodulatory properties.[10] In fact, between 2017 and 2021, 104 new triterpene glycosides have been found which possessed common activities such as cytotoxic, antitumor and neuroprotective.[11,12] Consequently, the aim of this research was to isolate the triterpene glycosides from the sea cucumber *Holothuria imitans* to evaluate its anticancer and neuroprotective activities in order to find secondary metabolites which may be potential alternatives for future drugs against cancer and neurodegenerative diseases.

MATERIAL AND METHODS

Animal material

Specimens of *Holothuria imitans* were collected from the coastal waters of the town of Mancora, Piura. The identification of the species was carried out by the fisheries laboratory of the National University of Trujillo.

Cell lines and media culture

The cell lines used for the assay were ATCC certified: VERO, MCF-7, MKN-28, A-549. Besides, it was used culture media D-MEM (Dulbecco's Modified Eagle Medium, Sigma) supplemented with 10% fetal bovine serum (FBS). DMEM/F12 medium supplemented with 0.1% fetal calf serum, sodium selenite 50 ng/mL, without antibiotic (Sigma).

Cite this article: Soto-Vásquez MR, Alvarado-García PAA, Jara-Aguilar DR, Rodrigo-Villanueva EM, Gavidia-Valencia JG, Beltrán-Alfaro IM, et al. Anticancer and Neuroprotective Effects of the Triterpene Glycosides From Sea Cucumber *Holothuria imitans*. Pharmacogn J. 2023;15(1):. 119-127

Extraction and isolation

The sea cucumbers were thawed with distilled water, cut length wise to remove the internal organs. The remaining body walls were lyophilized (4 Kg), pulverized and refluxed with 60% EtOH (6 L x 6, for 1 h), and then evaporated under reduced pressure in a rotary evaporator. The residue (500 g) was suspended in H_2O and placed in a DA-101 resin column (2 kg, 105 cm x 15 cm), using as eluents water (5 L), 70% ethanol (10 L) and 95% ethanol (5 L) respectively. The glycoside fractions were eluted with 70% ethanol. The different extracts were concentrated. The fraction of total crude glycosides (85 g) were separated by chromatographic column with silica gel (200-300 mesh, 3.0 kg) using as eluents $CHCl_3$-MeOH-H_2O (10:1:0.1, 9:1:0.1, 8:2:0.2, 7:3:0.5, 6:4:0.8) to give 4 fractions. (Fr. A-D). Fraction D was brought to CC (ODS, RP-C18), using 60:40 MeOH/H_20 as eluents to give 2 sub-fractions (D1 and D2). The D1 and D2 subfractions were purified by HPLC and using MeOH/H_20 (70:30) as the mobile phase and a flow rate of 1.5 mL/min.[13]

Acid hydrolysis of compounds

The compounds were refluxed with 1 mL of 2 mol/L trifluoroacetic acid at 120 °C for 2 h. The reaction mixture was evaporated to dryness and the residue was partitioned with CH_2Cl_2 and H_2O. The aqueous phase was concentrated under reduced pressure and 1 mL of pyridine and 2 mg of $NH_2OH{\cdot}HCl$ were added to the residue. This mixture was heated at 90 °C for 30 min. Then, 0.8 mL of Ac_2O were added and heating at 90 °C for 1 h. The resulting solution was concentrated and analyzed by GC-MS using peracetylated aldononitrile as standard.[13]

Anticancer activity

African green monkey kidney (VERO), and three cancer cell lines, gastric cancer (MKN-28), breast adenocarcinoma (MCF-7) as well as lung carcinoma (A-549) were used. The cell lines were maintained in D-MEM medium (Dulbecco's Modified Eagle Medium, SIGMA) supplemented with 10% fetal bovine serum (FBS). 100 units/mL of penicillin and 100 pg/mL of streptomycin were added to the culture medium to inhibit the growth of Gram negative and positive bacteria. Cell lines were incubated in a controlled atmosphere with 95% humidity, 5% CO_2 at 37°C/24h or until the formation of a monolayer. For the tests, the cells were obtained from cultures in exponential growth phase, with 0.25% trypsin, 0.53 mM EDTA and saline buffer (PBS).[14]

Cytotoxicity assay

Cell viability was evaluated using MTT assay (3-(4,5-dimethylthiazol-2-yl)-2.5 diphenyltetrazolium bromide) in 96-well cell culture plates. 2,500 cells/mL were added to each well using D-MEM medium supplemented with 10% fetal bovine serum, 100 units/mL of penicillin, 10 pg/mL of streptomycin and 0.25 µg/mL of amphotericin B. Then, cells were incubated for 24 h. After that, the triterpene glycosides added to the following concentrations:10, 20, 40, 80, 160 and 320 µmol/L and the plates were incubated again for 48 h (atmosphere 95% humidity and 5% CO_2 at 37 ºC). After this time, 10 µL of MTT solution in culture medium (5 mg/mL) were added to each well and incubated for four hours more. The supernatant was discarded, replaced with 100 µL of DMSO, and the absorbance was measured on a microplate spectrophotometer at 540 nm. Each test was performed by triplicate. 0.05% DMSO was used as a negative control and 10-hydroxycamptothecin (HCPT) as a positive control. The percentage (%) of cell viability was determined by the equation OD (sample) * 100 /OD (control).[14]

Neuroprotective activity

CAD and MO3.13 cell lines

In this research, Cath.a-differentiated (CAD) cell line and human glial (Oligodendrocytic) Hybrid cell line (MO3.13) were used as a cell model.

Cell cultures: CAD and MO3.13 cells were cultured in DMEM/F12 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin, using 75 cm^2 culture flasks, and at a density of $5x10^6$. The medium was replaced every three days and subcultures were made when obtaining a confluence of 80%. Then the CAD and MO3.13 cells were seeded in 96-well plates. The firsts one at a density of $7x10^3$ cells per well; while MO3.13 cells were seeded at a density of 45 x 10^3 cells per well. Subsequently, they were taken to an incubator at 37 °C and 5% CO_2. Finally, the CAD cells were differentiated for 48 hours; while MO3.13 cells for 72 to 96 hours.[15]

Cell count: Cells were detached and resuspended using 0.25% w/v Trypsin in a solution of PBS and EDTA 0.1% w/v. A sample of the suspension was taken and diluted in a solution 0.4% Trypan blue in a 1:4 ratio.10 µL was taken in a hemocytometer and counted the four quadrants at the corners of both graticules. This technique makes possible to differentiate living cells from dead ones, due to the fact that damage to its membrane allows the inclusion of the dye. Besides, the following formula was used:[15]

$$\text{Cells/mL} = \frac{\text{live cells counted}}{0.8\ mm^3} x \frac{1000 mm^3}{1mL} x\ dilution\ factor$$

Cytotoxicity test: Triterpene glycosides were subjected to the 50 lethal dose test in CAD and MO3.13 cell lines, and their viability was analyzed with MTT and WST-1 respectively.

Treatments with the neurotoxin C2-ceramide

The differentiated CAD cells were seeded in 96-well plates, at a density of $7x10^3$ per well and in DMEM/F12 medium supplemented with 0.1% fetal bovine serum, sodium selenite 50 ng/mL, without antibiotic. Triterpene glycosides were added in the appropriate doses prior to cytotoxicity studies and one hour later, the neurotoxin was added at a concentration of 25 µM for 6 hours and 10 µM for 24 hours. While the cells MO3.13 were seeded in a 96-well plate, at a density of $45x10^3$ per wellin DMEM medium supplemented with 100 nM PMA, with no antibiotic. Subsequently, the triterpene glycosides were added, as well as one hour later the C2-ceramide was added, at a concentration of 50 µM for 6 hours and 20 µM for 24 hours.[15]

Viability assays and cell death

The CAD cells were evaluated by the MTT assay, for which they were seeded in 96-well plates, at a density of $7x10^3$ per well, with a volume of 200 µL of means of differentiation. While the MO3.13 cells assay was performed of WST-1, for which the cells were seeded in a 96-well plate, at a density of $45x10^3$ and with 200 µL of differentiation medium. After the incubation time, 100 µL of culture medium was removed from each well, and 20 µL of the MTT solution (5 mg/mL) were added for CAD cells; as well as15 µL of the WST-1 solution for the cells MO3.13. The evaluation groups for both cell models of neuroprotection were control, ceramide, 2.5 µmol/L, 5 µmol/L and 10 µmol/L of triterpene glycosides, and 2.5 µmol/L, 5 µmol/L and 10 µmol/L of triterpene glycosides with ceramide. The evaluations were made at 6 and 24 hours respectively. The plates were incubated for 2 hours at 37 °C and 5% CO_2. Subsequently, the medium was removed, and 100 µL of DMSO was added, to dissolve the crystals. Finally, the absorbance was read with a spectrophotometer, at a wavelength of 490 nm for CAD cells and 450 nm for MO3.13 cells. All experiments were performed in triplicate.[15]

Statistical processing

The IC_{50} values of each triterpene glycosides were determined from the curves of concentration effect (% cell viability) by linear regression analysis with the use of the statistical package GraphPad Prism 5 (GraphPad Software, Inc.). For the neuroprotection test, the normality

test of D'Agostinos and Pearson, followed by one-way analysis of variance (ANOVA) followed by "Bonferroni's Multiple Comparison Test" ($p<0.05$) and analyzed by the computer program Software GraphPad Prism 5.

RESULTS

Structural isolation and elucidation two compounds were isolated from the sea cucumber (*Holothuria imitans*). Compound 1 was a colorless amorphous powder, while Compound 2 was a white crystalline solid. Both were positive with the Libermann-Buchard and Molish tests. Their molecular formulas were determined, from the combined analysis of HR-ESI-MS and ^{13}CNMR as $C_{54}H_{87}O_{27}SNa$ and $C_{54}H_{85}O_{26}SNa$ respectively, Both compounds presented pseudo-molecular ion peaks $[M + H]^+$ at m/z: 1246 (compound 1) and 1227 (compound 2) in positive ion mode and at m/z 1199 (compound 1) and 1190 (compound 2) $[M-Na]^-$ in negative ion mode. Likewise, fragments of the peak at m/z 1125 (compound 1) and 1107 (compound 2) $[M-OSO3Na^+Na-H]^+$, showed the presence of a sulfate group in both compounds, which were confirmed by IR to the bands of 1266 and 1074 cm^{-1} (compound 1) and 1258 and 1210 cm^{-1} (compound 2). The ^{1}H NMR and ^{13}C NMR spectra indicated the presence of an aglycone triterpene with seven methyls, an olefinic bond and a carbonyl lactone group in both compounds. The presence of the sugars *D*-xilose (Xyl), *D*-quinovose (Qui), D-glucose (Glc) and 3-*O*-Me-D-glucose (MeGluc) were determined in both compounds (1 and 2) in a ratio of 1:1:1:1, established by acid hydrolysis and the corresponding aldononitrile peracetates; which were analyzed by GC-MS. These compounds had a tetrasaccharide chain and a holostan backbone. The signals ^{1}H NMR and ^{13}C NMR, analyzed by 2D NMR indicated sugar residues in the form of pyranose. Based on the above information, compound 1 was identified as: 3-*O*-[3-*O*-methyl-β-*D*-glucopyranosyl-(1→3)-β-*D*-glucopyranosyl-(1→4)-β-*D*-quinovopyranosyl-(1→2)-4-*O*-sodium sulfate-β-*D*-xylopyransyl]-holosta-9(11)-ene-3β,12α-17α, 25α-tetrol and named as Scabraside D and compound 2 was identified as: 3-*O*-[3-*O*-methyl- β -*D*-glucopyranosyl- (1→3) - β -*D*- glucopyranosyl-(1→4) - β -*D*-quinovopyranosyl- (1→2) -4-*O*-sodium sulfate- β -*D*-xylopyranosyl]-22, 25-epoxy-9(11)-holostene-3 β,12 α -diol; called Fuscocineroside C (Table 1, Figure 1).

In Figure 2A, it is observed the percentages of cell viability of the two triterpene glycosides (Fuscocineroside C and Scabraside D) isolated from the sea cucumber "*Holothuria imitans*" against the VERO cell line, which decrease as the concentration of these increases. A higher percentage of cell viability was obtained at the concentration of 10 µmol/L (92.5% and 88.3% for Fuscocineroside C and Scabraside D respectively). Also in Figure 2B, IC_{50} values were higher in Fuscocineroside C (149.5 µmol/L) compared to Scabraside D (75.6 µmol/L).

Figure 3 shows the results of anticancer activity against gastric cancer cell lines (MKN-28), breast adenocarcinoma (MCF-7) and lung carcinoma (A-549). Fuscocineroside C shows greater anticancer activity than Scabracide D, for cell lines MKN-28 and A-549, getting a mean IC_{50} concentration of 0.92 µmol/L and 1.21µmol/L respectively, while the 10-hydroxycamptothecin control reached values 0.83 and 0.91 for MKN-28 and A-549.

Table 1: ^{1}H NMR (600 MHz, C_5D_5N) ^{13}C NMR (150 MHz, C_5D_5 N) of Scabraside D (1) and Fuscocineroside C (2).

	1		2			1		2	
Position	$\delta_H(J)$	δ_C	$\delta_H(J)$	δ_C	Position	$\delta_H(J)$	δ_C	$\delta_H(J)$	δ_C
1	1,39m,1.84m	36.5	1.76m,1.32m	36.6	**Xyl**				
2	1.88m, 2.07m	26.0	2.00m,1.87m	29.1	1	4.66d(7.3Hz)	106.2	4.56,d(6.4Hz)	105.1
3	3.12m	87.8	3.03m	88.9	2	4.04m	83.4	3.91m	85.1
4		40.0		40.0	3	4.28m	77.1	4.18m	75.2
5	0.97m	52.9	0.78m	52.9	4	5.12m	75.9	5.00m	78.5
6	1.51m, 1.77m	22.1	1.67,1.54m	25.1	5	3.80m,4.71m	66.3	4.58,3.60m	66.4
7	1.71m,1.73m	28.1	1.69m,1.45m	28.9	**Qui**				
8	3.35m	40.9	3.31m	40.2	1	5.02d(7.8Hz)	105.4	4.90d(7.6 Hz)	105.3
9		153.8		153.3	2	4.98m	76.3	3.80m	77.6
10		39.7		39.7	3	4.10m	74.0	6.00m	76.0
11	5.62(10.4Hz)	118.8	5.61(4.0Hz)	116.2	4	3.66m	88.0	3.59m	88.8
12	4.97m	70.9	4.45m	68.4	5	3.76m	73.7	3.60m	71.9
13		59.0		63.9	6	1.70d(6.0Hz)	20.0	1.58d(5.6Hz)	18.1
14		47.1		46.3	**Glc**				
15	1.80m,1.37m	38.0	1.56m,1.32m	37.4	1	4.96d(7.8 Hz)	105.2	4.86d(7.2Hz)	104.7
16	2.38m,2.96m	37.4	2.25m,1.94m	25.6	2	4.11m	75.4	3.99m	74.0
17		88.8	3.27m	47.6	3	4.23m	88.0	4.13m	88.0
18		175.6		176.9	4	4.12m	69.9	3.98m	69.6
19	1.37s	25.1	1.30s	22.5	5	4.02m	78.0	3.90m	77.8
20		86.9		84.1	6	4.47m,4.46m	61.8	4.31m,4.12m	63.9
21	1.74s	19.1	1.40s	22.0	**MeGluc**				
22	1.69m	38.0	4.14m	80.4	1	5.31d(8.0 Hz)	105.8	5.20(6.0Hz)	105.8
23	2.02m	28.4	1.90m,1.82m	27.4	2	4.05m	75.0	3.96m	75.0
24	1.61m	38.5	1.57m	38.7	3	3.70m	88.0	3.58m	87.9
25		81.8		81.2	4	4.09m	70.6	4.03m	71.9
26	1.18s	28.5	1.25s	28.7	5	3.99m	79.1	3.92m	78.2
27	1.16s	29.1	1.13s	29.1	6	4.20m, 4.46m	62.1	4.36,4.15m	64.2
28	1.04s	16.9	0.95s	16.7	**OMe**	3.86s	62.0	3.78s	62.7
29	1.22s	28.3	1.10s	28.6					
30	1.68s	20.6	1.19s	22.5					

Table 2: Cell viability of triterpene glycosides in CAD and MO3.13.

Triterpene Glycosides	6 hours			24 hours		
		CAD	MO3.13		CAD	MO3.13
	Concentration (µmol/L)	Cell viability $\bar{x}$ ± SD	Cell viability $\bar{x}$ ± SD	Concentration (µmol/L)	Cell viability $\bar{x}$ ± SD	Cell viability $\bar{x}$± SD
	0	100.0±0.7	100.0±0.5	0	100±0.8	100±0.7
	10	88±1.2	80±1.6	10	71±1.9	75±1.3
	20	70±0.9	69±0.8	20	50±1.5	60±1.1
Scabraside D	40	61±1.3	59±1.9	40	42±1.2	52±1.7
	80	52±0.8	52±1.3	80	32±0.7	47±1.2
	160	43 ±1 .6	40 ±1 .2	160	24±1.1	32±1.8
	320	33±1.2	30±1.5	320	10±1.8	20±1.6
	0	100±0.5	100±0.5	0	100±0.4	100±0.4
	10	91±1.4	92±1.3	10	79±1.8	82±1.9
	20	80±1.2	87±1.1	20	64±1.7	73±1.5
Fuscocineroside C	40	72±0.9	80±0.5	40	55±1.5	69±1.2
	80	65±1.5	75±1.9	80	50±1.3	60±1.1
	160	59±0.8	69±1.2	160	41±0.8	56±1.4
	320	50±1.4	60±0.8	320	31±1.4	44±1.7

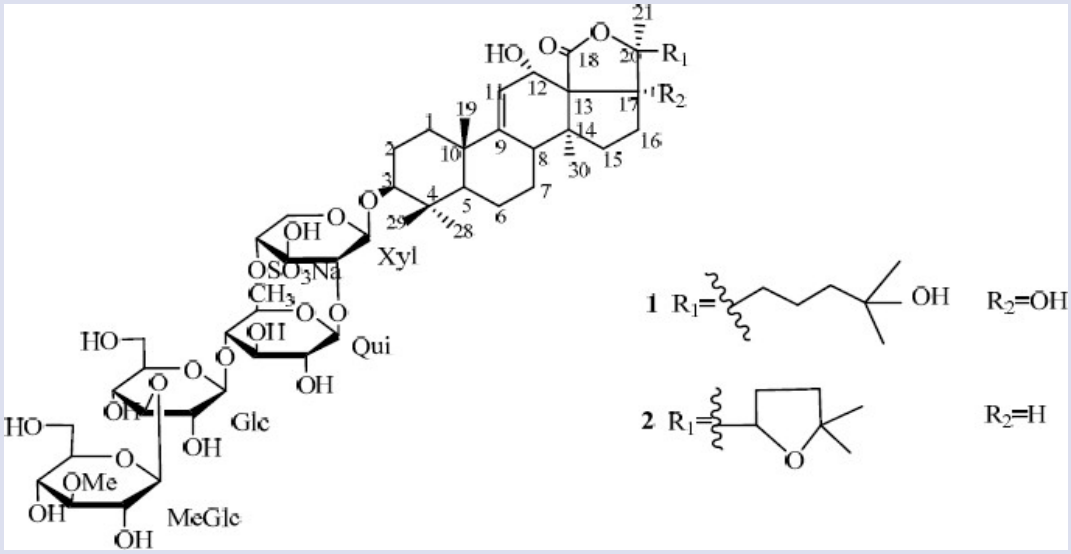


Figure 1: Structure of triterpene glycosides: Scabraside D (1), Fuscocineroside C (2).

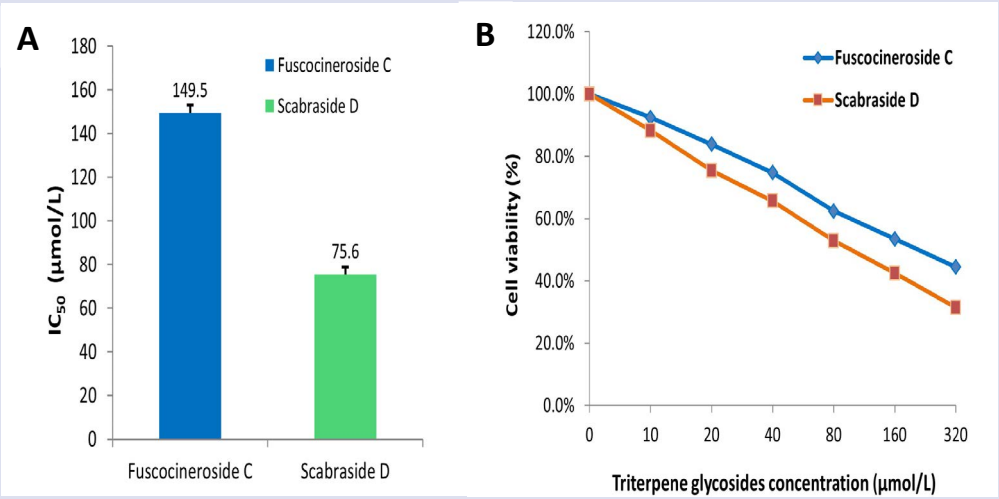
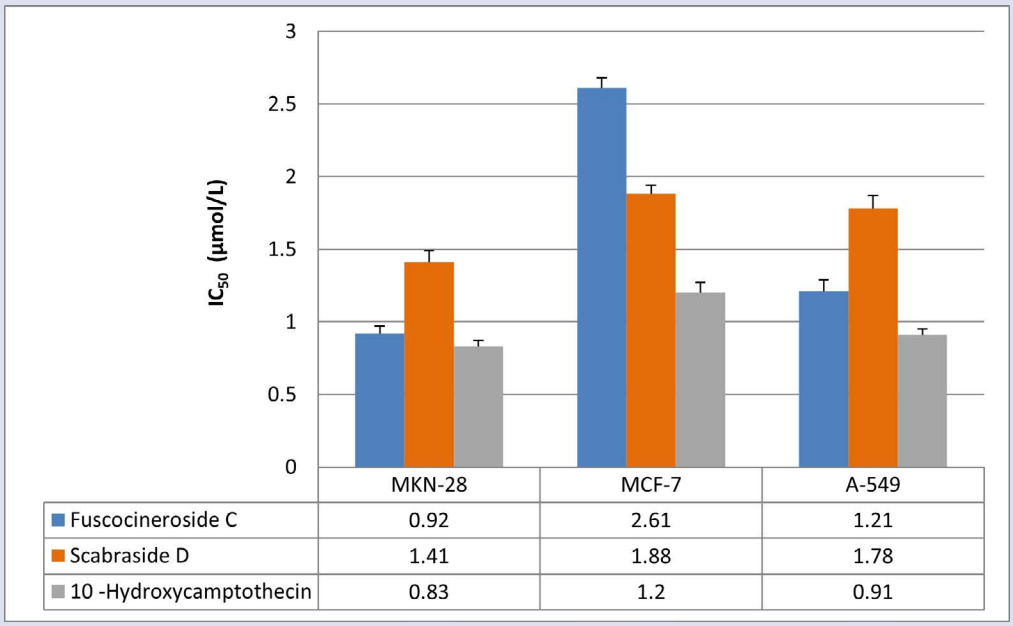


Figure 2: Cytotoxic activity of triterpene glycosides against isolated VERO cell line of the sea cucumber "*Holothuria imitans*". A) Cell viability (%), B) IC_{50} values.



	MKN-28	MCF-7	A-549
Fuscocineroside C	0.92	2.61	1.21
Scabraside D	1.41	1.88	1.78
10 -Hydroxycamptothecin	0.83	1.2	0.91

Figure 3: IC_{50} values against MKN-28, MCF-7 and A-549 cell lines.

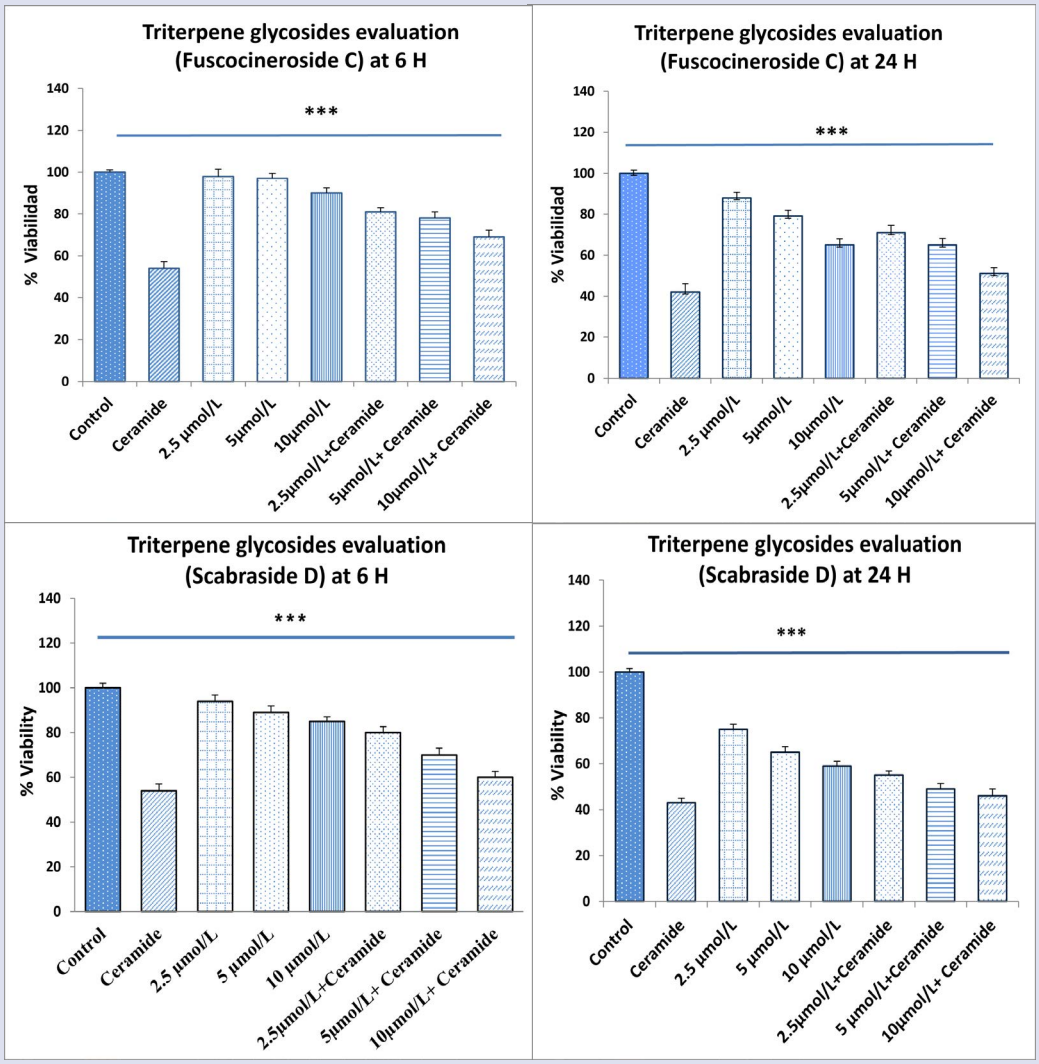


Figure 4: Neuroprotective effect through mitochondrial viability in CAD cells, of triterpene glycosides against C-2 ceramide. ***p<0.05

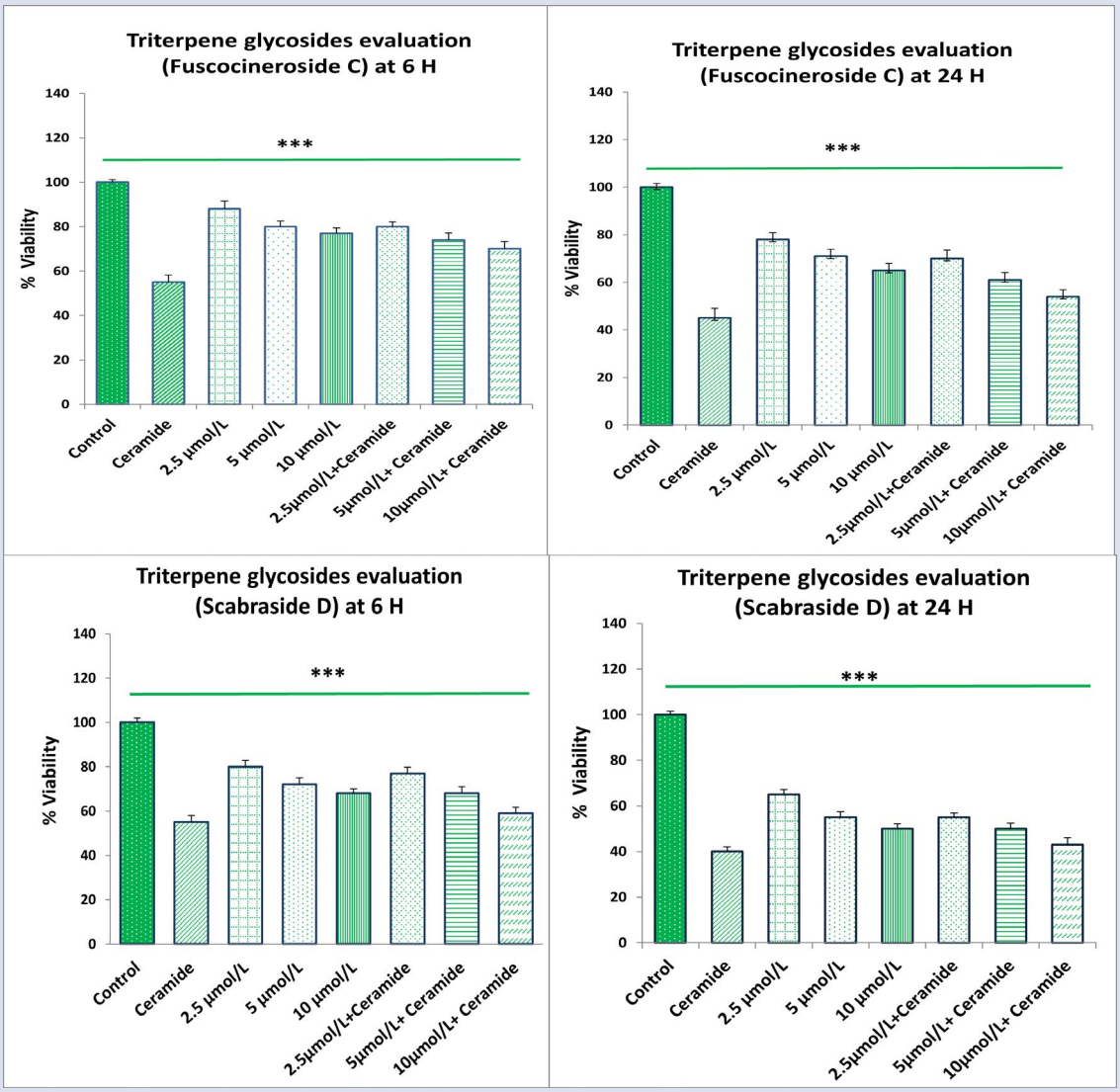


Figure 5: Neuroprotective effect through mitochondrial viability in MO3.13, of triterpene glycosides against C-2 ceramide. ***p<0.05

In table 2, the results of the cell viability assays of triterpene glycosides in CAD and MO3 cells are expressed at 6 and 24 hours of treatment, where the concentration of 10 µmol/L, for both triterpene glycosides presented the higher percentages of cell viability, exceeding 70% in both cell lines.

In figure 4 the results of the neuroprotective effect through mitochondrial viability in CAD cells are observed, where Scabraside D and Fuscocineroside C have greater mitochondrial viability at the dose of 2.5 µmol/L, at 6 and 24 hours of treatment; showing Fuscocineroside C greater neuroprotective effect in comparison to Scabraside D.

Statistically significant results were obtained $p<0.05$, by means of the one-way ANOVA test and the Bonferroni multiple comparison test.

Besides, in figure 5 the results of the neuroprotective effect through mitochondrial viability in MO3.13 cells are observed, where Scabraside D and Fuscocineroside C have greater mitochondrial viability at the dose of 2.5 µmol/L, at 6 and 24 hours of treatment; showing Fuscocineroside C greater neuroprotective effect than Scabraside D. Statistically significant results were obtained $p<0.05$, by means of the one-way ANOVA test and the Bonferroni multiple comparison test.

DISCUSSION

The marine environment offers a rich source of natural products with potential therapeutic applications,[16] where sea sponges and sea cucumbers are the richest in natural compounds.[17] Thus, in this work, two triterpene glycosides were isolated by chromatographic techniques from the extracts of the sea cucumber (*Holothuria imitans*). These compounds were identified as Fuscocineroside C and Scabraside D using ^{1}H NMR and ^{13}C NMR analysis. In this sense, Scabraside D is very similar to the aglycone Echinoside A and Holothurin A3 but differs from them in C-25 and C-22 respectively;[18] likewise, Fuscocineroside C is similar to Holoturin A.[19] These compounds have also been found in other species of sea cucumbers from genus *Holothuria* and showed pharmaceutical potential.[20,21]

In the present work, the isolated compounds presented low cytotoxicity against normal VERO cells with IC_{50} of 149.5 µmol/L and 75.6 µmol/L respectively. Regarding the anticancer activity, these compounds showed a high cytotoxic activity against the cell lines MKN-28 (gastric cancer), MCF-7 (breast adenocarcinoma) and A-549 (lung carcinoma) with IC_{50} values of 0.92 to 2.61 µmol/L; where Fuscocineroside C presented higher anticancer activity compared to Scabracide D, for the cell lines MKN-28 and A- 549 with a mean IC_{50} concentration of 0.92

µmol/L and 1.21 µmol/L respectively; while 10-hydroxycamptothecin, an analog of the campothecin alkaloid, which is an antitumor, obtained an IC_{50} of 0.83 and 0.91 for MKN-28 and A-549 respectively. In fact, the IC_{50} value of Fuscocineroside C is close to the IC_{50} value of the control drug in the gastric cancer cell line which is in accordance with other investigation that also show the cytotoxic effect of Fuscocineroside C in other cell lines such as human promyelocytic leukemic cell line (HL-60) and human hepatoma cell line (BEL-7402).[22]

Besides, Scabraside D has shown to induce the apoptosis in HepG2 cells, derived from human hepatoblastoma; as well as human cholangiocarcinoma cells.[21] Likewise, different authors affirm that the membranolytic activity of triterpene glycosides, a mechanism of sea cucumbers against predators, is the probable cause of the anticancer effects of these compounds, which is closely related to their structure, where the triterpene lanostane with lactone and the C3-linked sugar chain of the aglicone are responsible for the significant cytotoxic activity.[19,23] Thus, the difference in the chain sides of these metabolites may explain the differences in IC_{50} values.

On the other hand, regarding to the neuroprotection assay, cell viability tests of triterpene glycosides were performed in CAD and MO3.13 cells, at 6 and 24 hours of treatment, determining the concentration of 10 µmol/L to be worked in the mitochondrial viability assays, because this, for both compounds, represented the highest percentages of cell viability. As a result, Scabraside D and Fuscocineroside C have greater mitochondrial viability at the dose of 2.5 µmol/L compared to C-2 ceramide, at 6 and 24 hours of treatment in both cell lines. C-2 ceramide was chosen as neurotoxic, because it has numerous effects on the mitochondria such as producing reactive oxygen species, collapse of the mitochondrial membrane potential, among others.[15] However, Fuscocineroside C was the compound that obtained a greater neuroprotective effect than Scabraside D. These outcomes are in agreement with other investigation where extracts from *Holothuria scabra*, rich in triterpene glycosides such as Scabraside A, B and D as well as Fusconeroside, showed neuroprotective and neurorestorative effects, finding that the possible mechanism of action might be due to the fact that these compounds prevent mitochondrial membrane depolarization, in addition to stimulate the production of tyrosine hydroxylase, essential enzyme for the normal functioning of the nervous system.[23] Besides, other different studies have demonstrated the neuroprotective effects of triterpene glycosides; these compounds being able to stimulate neurogenesis and thus combat neuronal damage caused by neurodegenerative diseases.[25]

CONCLUSION

The triterpene glycosides Fuscocineroside C and Scabraside D isolated from the sea cucumber *Holothuria imitans* possess anticancer and neuroprotective potential and may be considered promising active principles for the development of new drugs.

ACKNOWLEDGEMENTS

The authors thank to V Call for Science and Technology Projects of the National University of Trujillo with public funds from CANON 2021, modality 4 (PIC N° 20-2021).

CONFLICTS OF INTEREST

All authors have no conflicts of interest to declare.

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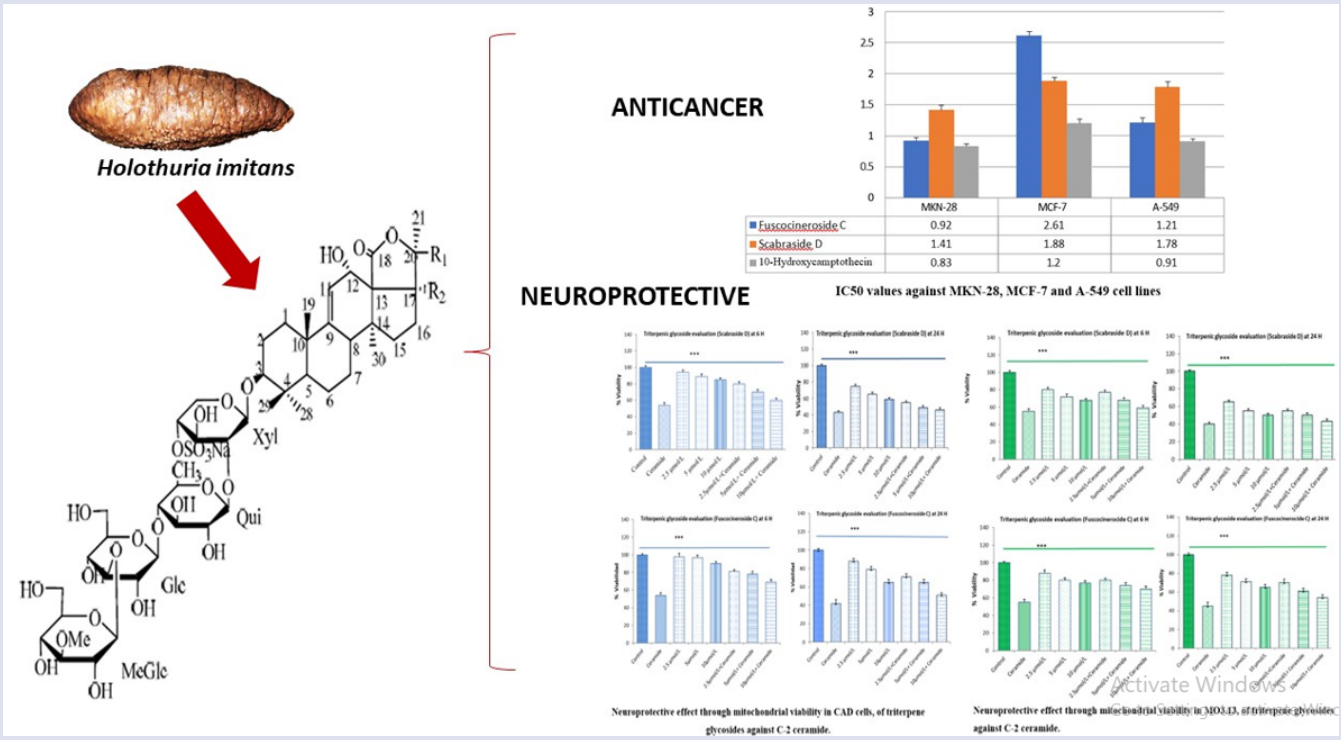
GRAPHICAL ABSTRACT



ABOUT AUTHORS

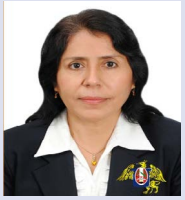
Dra. Marilú Roxana Soto-Vásquez: Doctor in Pharmacy and Biochemistry. Professor of pharmacognosy and phytochemistry at Faculty of Pharmacy and Biochemistry of Universidad Nacional de Trujillo. Develops Research in Natural Products with antiprotozoal, antitumor, antimicrobial activities, and Complementary and Alternative Medicine.

M.Ed. Paul Alan Arkin Alvarado-García: Master of Education. Professor at the Medicine School of Universidad César Vallejo. Develops research in Mental health problems, Complementary and Alternative Medicine, and natural products applicated to mental disorders.

Dr. Demetrio Rafael Jara-Aguilar: Doctor in Pharmacy and Biochemistry, professor at National University of Trujillo, Develops Research in Natural Products and Chemical Physical Analysis of food.

Dra. Elda Maritza Rodrigo-Villanueva: Doctor in Pharmacy and Biochemistry, professor of the chairs of Investigation methodology. Develops Research in Natural Products.

Dr. José Gilberto Gavidia-Valencia: Doctor in Pharmacy and Biochemistry, professor at National University of Trujillo, section Natural Products. Develops Research in Natural Products, functional foods and nutraceuticals.



M. Pharm. Iris Melina Alfaro-Beltrán: Master of Pharmacy. Specialist in Toxicology. Professor at Pablo Casals Institute. Develops research in natural products and nutrition.

Pharm. Bertha Mirella Alfaro-Ttito: Chemical pharmaceutical, student at Postgraduate School of National University of Trujillo, section Natural Products. Research Assistant in Natural Products.

Cite this article: Soto-Vásquez MR, Alvarado-García PAA, Jara-Aguilar DR, Rodrigo-Villanueva EM, Gavidia-Valencia JG, Beltrán-Alfaro IM, et al. Anticancer and Neuroprotective Effects of the Triterpene Glycosides From Sea Cucumber *Holothuria imitans*. Pharmacogn J. 2023;15(1): 119-127.