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Hemolytic and cytotoxic effects of saponin like compounds isolated from Persian Gulf brittle star (*Ophiocoma erinaceus*)

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ABSTRACT

Objective: To isolate and characterize the saponin from Persian Gulf brittle star (*Ophiocoma erinaceus*) and to evaluate its hemolytic and cytotoxic potential.

Methods: In an attempt to prepare saponin from brittle star, collected samples were minced and extracted with ethanol, dichloromethane, *n*-buthanol. Then, concentrated *n*-butanol extract were loaded on HP-20 resin and washed with dionized water, 80% ethanol and 100% ethanol respectively. Subsequently, detection of saponin was performed by foaming property, fourier transform infrared spectroscopy and hemolytic analysis on thin layer chromatography. The cytotoxic activity on HeLa cells was evaluated through 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT) assay and under invert microscopy.

Results: The existence of saponin in *Ophiocoma erinaceus* were approved by phytochemical method. The presence of C-H bond, C-O-C and OH in fourier transform infrared spectrum of fraction 80% ethanol is characteristic feature in the many of saponin compounds. Hemolytic assay revealed HD_{50} value was 500 μg/mL. MTT assay exhibited that saponin extracted in IC_{50} value of 25 μg/mL inducsd potent cytotoxic activity against HeLa cells in 24 h and 12.5 μg/mL in 48 h, meanwhile in lower concentration did not have considerable effect against HeLa cells.

Conclusions: These findings showed that only 80% ethanol fraction Persian Gulf brittle star contained saponin like compounds with hemolytic activity which can be detected simply by phytochemical that can be appreciable for future anticancer research.

1. Introduction

Natural products isolated from marine environment is well known for its pharmacodynamic protential in diversity of disease treatments such as cancer or inflammatory condition. In folk medicine, marine organisms was applied for wound healing and amelioration of lesion[1]. Biodiversity of marine ecosystem has obtained a broad range of highly biological compound from marine source which have useful for drug discovery. To date, different groups of research have evaluated biomedical potential of appreciable marine natural product in many aspects[2].

Class Ophiuroidea (brittle star) belongs to echinoderms and is famous for its extensive repairing capacity specially in arm regeneration. Wang *et al.* could isolate terpens, sulfated sterols phenyl propanoides from *Ophioplocus japonicus* (*O. japonicus*) as bioactive substance; some of these isolated metabolite from *O. japonicus* have exhibited antibacterial activity against microbial strains while other component displayed moderate or high cytotoxicity[3]. A few studies were reported about the biological activity of brittle star species. However antiviral effect of brittle star

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demonstarated that sterols, steroidal glycosides, carotenoid sulfates and naphtoquinone are major in bioactive metabolites obtained from brittle star which are responsible for life maintenance in the worldwide. These compounds may prepare them for biomedicine application[4].

Saponins and glycosylated ceramides obtained from echinoderms are greatly responsible for their biological activity[5]. Due to high frequency of triterpenoid saponin in cultivated crops, most of research were focused on chemopreventive and cytotoxic activity of triterpenoid saponin and correlated with steroidal saponin which is rich in herbs available in a few documentations[6]. In starfish, steroidal glycoside (responsible for numerous biomedicine properties such as cytotoxic effect) were dominant and classified into three main class: asterosaponins, cyclic steroidal glycosides and glycosides of poly hydroxylated steroids. Generally, there is structural diversity of saponin between echinoderms significantly. Isolated saponins possessing hemolytic activity in starfish mostly have been obtained sterol derivatives whereas terpenoid saponins mainly have isolated from sea cucumber[5].

Saponins as secondary metabolites are amphiphilic glycol conjugates mainly originated from plant extract but it was showed these substances was abundantly found in marine animals specially sea cucumber and sea star[7]. Prominent structure of saponin is composed aglycones and sugar side chaines. According to structure of aglycon, saponins were classified into triterpenes and steroids. In terrestrial plants triterpenoid saponins are more widely distributed than steroidal saponins[8].

The first characterization of saponin in plant extract or marine extract is strong foam forming ability in aqueous solution. Amphiphilic nature of saponins (*i.e.* presence of a lipid-soluble sapogenin moiety linked to water soluble sugar chains) enable saponins as surface active compounds with foaming feature to interact with cell membrane[9].

Due to surface activity of saponins these compound have been able to disrupt cell membranes (*i.e.* form complexes with cell membrane cholesterol) which this lytic action on erythrocytes membrane utilize as saponin detection[10].Most of the researches to date were indicated, anti microbial, anti inflammatory, anti tumor, anti diabetic and anti fungal, and adjuvant activity of varied saponins[11]. There are some conflicted information about drug application of saponins. Lacaille-Dubois *et al.* 1996, were indicated glucocorticoid like effect of saponins associated them as precursor of therapeutic medicine such as contraceptive estrogen and cortisone[12]. For instance, Mats in 1990 were exhibited (triterpene glycosides holotoxin A1,B1) showed contraceptive activity[13]. To date, several triterpenoid saponin were extracted from sea cucumber and were examined their physiologically active potential broadly among echinoderms in biomedicine[14].

Due to less evidences related to saponin existence in Persian Gulf echinoderms, particularly Ophiuroidea, the present study aimed to firstly demonstrated existence of saponin in Persian Gulf brittle star *Ophiocoma erinaceus* (*O. erinaceus*) and evaluated hemolytic and cytotoxicity of these compounds on HeLa cervical cancer cells.

2. Materials and methods

2.1. Reagents and chemicals

Brittle star species *O. erinaceus* were collected from rocky intertidal flats of Qeshm island in Persian Gulf. Ethanol, dichloromethane, *n*-butanol, chloroform, ascetic acid were purchased from Merk, Germany. HP-20 resin were prepared by Sigma, USA. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT) and quillaja saponin were purchased from Sigma, USA. Pre-coated silica-gel-60-F254 plates detected at 254 nm was purchased from Merck, Germany.

2.2. Prepararation and extraction of saponin fraction from Persia Gulf brittle star

At first, morphometric analysis of *O. erinaceus* was performed at Research Center Applied Biology of Mashhad Islamic Azad University. Extraction of saponin like compounds from *O. erinaceus* were prepared according to the method described by Hu *et al.*[15].

In brief, to remove impurities samples were washed with water, and grinded into small pieces. Dried brittle stars soaked in ethanol at room temperature for 3 d at dark then was extracted three times by refluxing with ethanol for 3 h. In next step, cooled extract was filtered by Whatman paper 11 μm and evaporated under reduced pressure in Heidolph, Germany. Thereafter concentrated extract was defatted with dichloromethan or chloroform/water for 6 h. After desired time, upper fractionation (water layer) was collected and extracted with *n*-butanol. The organic layer was evaporated to yield *n*-butanol extract. At last step, extracted residue was dissolved in distilled water and was loaded on Diaion HP-20 resin column and washed with dieonized water, 80%

ethanol and 100% ethanol respectively to obtained fraction 50, 80, 100. At the end, fractionated extract was condensed, freeze dried and stored at −20 °C.

2.3. Phytochemical screening

To identify the existence of saponins in obtained *n*-butanol extract was used the froth formation. For performance of this method, a little volume of various fractionation of *n*-butanol extract were dissolved in distilled water and test tube was shaked strongly for 1 min. Continious appearance of foam in mixture indicated the presence of saponin.

2.4. FTIR spectroscopy

To identify the functional groups of the active compound, infrared spectroscopy in the region of infrared radiation was utilized. To do this work, dried fraction 80 containing saponin and standard saponin were ground to quite powder with mesh sieve and mixed 1/1 with vacuum dried KBr powder to make compressed pellet with subsequent recording of infrared spectrum using Perkin Elmer spectrometer. Spectra were recorded in the absorbance mode from 4 000 to 400 cm^{-1} on a Perkin Elemer Paragon 1 000 fourier transform infrared spectroscopy (FTIR) spectrometer (USA).

2.5. TLC analysis and qualitative detection with ethanol: sulphuric acid reagent

Thin layer chromatography (TLC) has been used as simple and rapid technique to identify isolated components from natural substances. To speed up analysis and comparison of many samples simultaneously cause extensive application of this tool for confirmation of the presence of some natural products such as saponins in phytochemical analysis. TLC analysis was carried out on pre-coated TLC paper. In order to detection presence of saponin in our fractions, on 2 silica gel coated TLC paper 10 μL of samples and standard saponin were directly spotted with pipette and kept dry for 10 min. Then TLC papers eluted in a solvent comprising the mixture of *n*-butanol: water: acetic acid in the ratio of (84:14:7) as special solvent system. When solvent system reached to paper upper line, TLC papers was removed from chamber and dried at room temperature. Saponin qualitative identification was performed by spraying with ethanol: sulphuric acid. For this stage, ethanol: sulphuric acid (90:10) was sprayed on developed TLC paper and heated to appear color and separated bands[16].

2.6. Qualitative erythrocyte hemolysis assay

Hemolytic activity is considered as semi quantitative test for validation of some saponins. To perform this process red blood cells were obtained from rat. Blood was collected with 5 mL syringe which was filled with sodium citrate as anticoagulant and was centrifuged at 2 300 r/min for 5 min and supernatant was discarded. The obtained pellet was diluted in phosphate buffered saline (PBS) (pH=7) to concentration 3% (v:v). Then the suspension of red blood cells were spilled in a tray and prepared TLC paper was suspended horizontally for 20 seconds. Next, TLC paper removed from red blood cells suspension and kept as vertically 30 seconds. For removing excess blood, TLC paper was soaked 30 seconds and then kept vertically 30 min additionally. A good contrast between the background and the spots developed during this period and the chromatogram was ready for documentation. The data were expressed as hRst values:

$$\text{hRst} = \frac{\text{Distance of sample spots from start point}}{\text{Distance of solvent movement from start point}} \times 100$$

Whitening spots on TLC plates was prominent indicator of the presence saponins.

2.7. Quantitative hemolytic activity assay

The hemolytic activity of *O. erinaceus* saponin compounds was measured on the basis of method Hassan *et al.* Briefly, Fresh blood was obtained from rats by cardiac puncture and collected using syringe filled with sodium citrate and placed in ice. Separation of rat erythrocyte were performed by centrifugation at 4 000 r/min for 5 min. To decolourize obtained pellet was washed three times with PBS and 2% erythrocyte suspension were used for assessment hemolytic activity. Dissolving of fraction 50, 80, 100 was prepared in 1 mL PBS and 96 well plate was used for evaluation of hemolytic activity. Six rows (1 row equal to 8 well) were considered for this step. In this experiment, PBS acted as negative control and standard saponin (quillaja saponin) used as positive control. Firstly, 100 μL PBS were added to 5 rows which 1 row contained PBS alone as minimal hemolytic control. One row considered maximal hemolytic control that 100 μL standard saponin and 100 μL of 2% red blood cell was added. Four rows contained 100 μL fraction of 50, 80, 100 in addition to 100 μL of 2% erythrocyte. Then plate was covered with parafilm and incubated 3 h at room temperature. Finally, free hemoglobin was measured spectrophotometrically at 650 nm as efficient wavelength.

2.8. Cell cytotoxicity assessment

HeLa cervix cancer cells at the initial density of 10^4 were cultivated in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS) and 1% antibiotic in flat-bottom 96 well plate for 24 h and incubated with different concentrations of brittle star saponin like compounds for 24, 48, 72 h in 5% CO_2, humidified incubator at 37 °C. After treatment period, 30 μL MTT added to wells and incubated in 37 °C for 4 h. after decanting media containing MTT formazan crystal were dissolved with dimethyl sulfoxide. Percent of cell viability and cell growth arrest in experimental groups were measured at the absorbance of 570 nm.

2.9. Statistical analysis

Data analysis was performed using Student's *t* test to evaluate the statistical significance of experimental groups. The results were recorded as means±SD. $P<0.05$ was consider significance.

3. Results

3.1. Phytochemical analysis

The analysis confirmed the presence of saponin in Persian Gulf *O. erinaceus*. The persistent foam formation exhibited only in 80% ethanol fraction between all fraction of *n*-butanol extract shown in Tables 1 and 2.

Table 1

Erythrocyte lysis effect of various fractions of Persian Gulf brittle star (mean±SD).

Groups	Absorbance (optical density)
Negative control	1.500±0.025
Standard saponin	0.290±0.010
Water fraction	1.530±0.007
50% Ethanol fraction	1.320±0.070
80% Ethanol fraction	0.230±0.007
100% ethanol fraction	1.400±0.040

Table 2

Hemolytic activity of different concentration of 80% ethanol fraction.

Groups	Absorbance (optical density)
PBS	1.500±0.025
Standard saponin	0.290±0.010
1000 μg/mL	0.080±0.004
750 μg/mL	0.160±0.001
500 μg/mL	0.510±0.010
250 μg/mL	1.080±0.030
125 μg/mL	1.250±0.090
62.5 μg/mL	1.300±0.020
31.25 μg/mL	1.320±0.040

3.2. Confirmation of saponin existence by IR analysis

The FTIR spectrum of fraction 80% ethanol revealed the presence of broad and strong signal of hydroxyl group (3398.78 cm^{-1}), C-H (2851.54 cm^{-1}), signal olefinic (C=C) (1673.33 cm^{-1}), strong absorption signals sulfate group (C-O-C) noted at 1213.97 cm^{-1}, 1055.14 cm^{-1} were assigned in Figure 1.

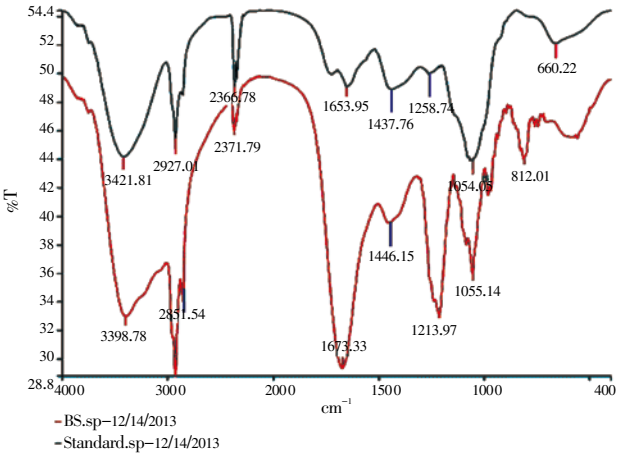

Figure 1. FTIR spectrum of brittle star saponin like compounds in the 4000-400 cm^{-1} region.

Infrared absorption spectrum offraction in 80% ethanol approximately indicated same pattern of standard saponin (Quillaja saponin) and obtained absorptions are characteristic of saponin structure.

3.3. Qualitative hemolytic study of brittle star saponin like compounds

The presence of white spots at dried TLC paper soaked in red blood cell suspension which is hallmark of saponin existence and characterized saponin structure just observed in fraction 80% ethanol and standard saponin which is verified FTIR analysis (Figure 2).

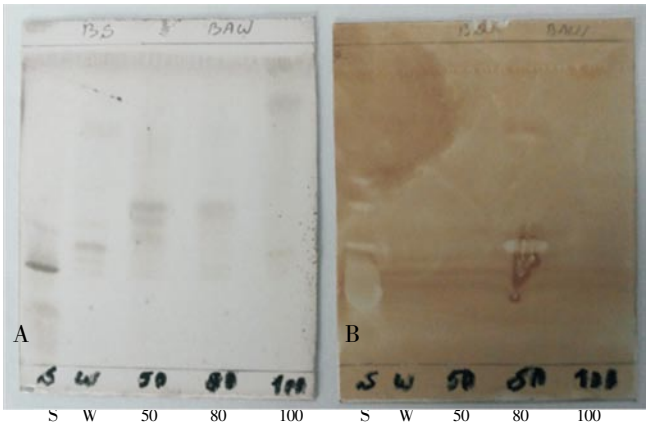

Figure 2. % hRst hemolysis TLC fraction 80% ethanol equal to 35%.

A: TLC were observed obviously glycoside band in saponin standard, water fraction, 50% ethanol, 80% ethanol, 100% ethanol (left to right); B: TLC were observed clearly band just in standard saponin and 80% ethanol. S :standard saponin; W: water fraction; 50: 50% ethanol fraction; 80: 80% ethanol fraction; 100: 100% ethanol fraction. BAW: Mobile phase and BS: Brittle star.

3.4. Semi quantitative hemolysis assessment of brittle star saponin like compounds

This study investigated that the hemolytic activity of different fraction obtained from arms of *O. erinaceus*. It is recognized that among different fraction, erythrocyte treated with various fractionation of *n*-butanol extract (50% ethanol, 80% ethanol, 100% ethanol) and water fraction, optical density of 80% ethanol fraction was similar to standard saponin (quillaja saponin) and indicated high hemolytic activities compared with other fraction. No considerable hemolytic properties was shown at 50% ethanol, 100% ethanol and water fraction as compared to reference saponin. Consequently, merely 80% ethanol fraction among all fractions contain capacity of red blood cells lysis and HD_{50} was calculated as 500 μg/mL 80% ethanol fraction.

3.5. Antiproliferative effect of brittle star saponin like compounds on HeLa cell growth

The results indicated that crude saponin extracted from Persian Gulf brittle star moderately decreased tumor cell viability in a dose and time dependent manner. In the range of upper concentrations (up to 25 μg/mL brittle star saponin) the growth of HeLa tumor cells was decreased vigorously in 24 h treatment. IC_{50} was observed at a concentration of 25 μg/mL experimental group 24 h treatment. As shown in Figure 3, *O. erinaceus* saponin extract reduced cell proliferation in a dose, and time dependent manner and 12.5 μg/mL concentration was evaluated as IC_{50} in 48 h incubation with saponin like compounds. The 50% inhibition rates of brittle star saponin like compounds against HeLa human cervix cancer cell growth were exhibited when treated with 25 μg/mL, 12.5 μg/mL respectively in desired time.

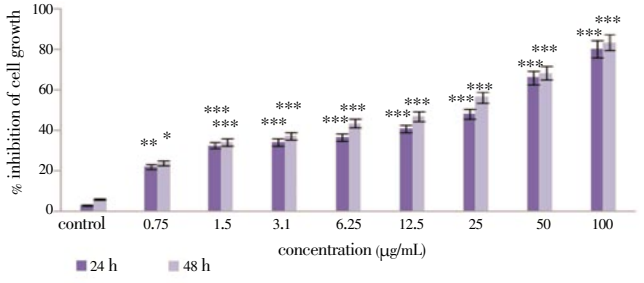

Figure 3. The cytotoxic effect of brittle star saponin like compounds on cell growth of HeLa cell line for 24 h and 48 h after exposure to different dosage of saponin like compounds.

Mean±SEM. **P*<0.05, ***P*<0.01 and ****P*<0.001 were considered significant. As shown in the figure, saponin like compound extracted inhibited HeLa cell proliferation as time and dose dependent.

4. Discussion

Marine ecosystem provides rich sources of bioactive natural products with structurally diverse which is crucial for improving human health. Isolation and identification of novel metabolite from marine organisms have created efficient drugs with valuable therapeutic and pharmaceutical potential[2]. Because saponin containing plants in terrestrial ecosystem possess important beneficial features in biomedicine and saponin is considered as major constituents of some herbal drugs, investigation in related to their existence in marine environment have been attractive for scientists.

Persian Gulf have been famous as main resource of unknown potentially bioactive compounds that is considered as habitat of several marine organism. Fatemi *et al.* in 2007 were collected and identified species of Ophiuroidea in Qeshm Island (Persian Gulf) using several methods.

Their findings showed *O. erinaceus* and *Ophiocoma scolopendrina* belonging to a single family Ophiocomidae were existed in Qeshm island in Persian Gulf[17]. Keshavarz *et al.* in 2012 investigated echinoderms fauna in intertidal zone of Southern Oli village coast and quatified 5 species of echinoderms on the basis of valid literatures. Their study have been indicated brittle stars (*O. erinaceus*) were dominant in sublittoral zone. Therefore, Persian Gulf species of *O. erinaceus* with long body, thick spines on arms, shorter arms compared to arm tips and diverse color as common species were used in our experiments[18].

In present study, confirmation of saponin accomplished without expending expensive and time consuming chemical methods. Stable froth formation as screening phytochemical method of saponins validated the FTIR analysis. Hemolytic assay established the presence of saponin quantitatively and qualitatively in Persian Gulf brittle star. MTT assay revealed a moderate inhibitory effect on viability of cervix tumor cells under treatment of saponin fraction obtained from Persian Gulf brittle satr with IC_{50} values of 25, 12.5 μg/mL in 24, 48 h, respectively.

The rapid extraction and simple identification of saponins can mainly create application of many of these compounds in clinical trials. Quantitation of saponins can be achieved by several methods[19]. Foamnig property of saponin in aqueous solution is simple, fast, and economical method for confirmation of saponin presence[9]. FTIR technique is not expensive, short time and highly specific method for saponin identification. Recognition of saponin in brittle star were confirmed by infrared absorptions recorded. FTIR spectrum of the reference sample powder and *n*-butanol

extracts (fraction 80% ethanol) indicated characteristic absorption peaks of saponin. C-O-C band (1055.14 cm^{-1}) is oligosaccharide linkage absorption to aglycon part of saponin. In experimented brittle star glycon part through ether linkage linked to sapogenin.

Existence of -OH, -C=O, C-H, and C=C bands in absorptions peak of FTIR spectrum was characteristic of saponins[19]. The C-O-C absorptions indicated glycoside linkages to the sapogenins. Therefore, FTIR spectra represented indicative sign of presence saponin like compounds in Persian Gulf brittle star. Some studies were used FTIR for saponin characterization. For instance, Kareru *et al.* in 2008 performed FTIR spectroscopy for direct identification of saponin in herbal drug[16].

The unique chemical structure of saponins is related to their hemolytic effects. Pore formation and cell permeabilization of saponins cause alteration of negatively charged carbohydrate portions on the erythrocyte cell surface which lead to lyse erythrocyte. Complexity of sugar moiety and the number of side chain in saponin have influenced hemolytic activity[10]. In fact, interference with cellular membranes of saponin cause destabilization of erythrocyte membrane which this specific feature was used to quantify drug for along time. Hemolytic activity have been used for development of saponin determination. Hemolytic assay can be performed both quantitative and qualitative methods[16].

In 2013 Inalegwu evaluated hemolytic activities aqueous extracts of leaves of *Tephrosia vogelii* and purified saponins and then reported these substance possess identical hemolytic effect on human red blood cells[20].

Therefore Sun in 2008 assessed hemolytic activitiy of saponins isolated from the rhizoma of *Anemone raddeana* and demonstrated saponins extracted exhibited a low hemolytic effect, with 16.50% and 3.56% at the concentrations of 500 and 250 μg/mL, respectively which resemble to our data in hemolytic activity of saponin[21].

In another study (Hassan *et al.*) saponin rich extracts isolated from guar meal were examined for hemolytic effect using quantitative method and concluded that only 100% MeOH fraction showed hemolytic activity while our extraction methods showed 80% EtOH fraction possess hemolytic effects[22].

In 2012 Sharma *et al.* reported a simple and convenient technique for detection of saponin which without usage of corrosive chemical can recognize saponin by combination thin layer chromatography and hemolytic assay qualitatively[23].

An enormous number of reports were evaluated chemopreventive role and cytotoxicity of saponin on various cancer cell line *in vitro*. There are apparent structure - activity relationships between structure and the cytotoxicity in both steroid and triterpenoid saponins.

Xu *et al.* in 2012 showed anti tumor effect of saponin isolated from *Pulsatilla chinensis* in human liver tumor 7402 cells *in vitro* and *in vivo* in a dose dependent manner with IC_{50} of 150.35 μg/mL that demonstrated strong chemopreventive effect of saponin extracted. Obtained IC_{50} saponin extracted from brittle star was approximately 50 μg/mL that it established efficacy of isolated substance against cervical cancer cells[24].

Tong *et al.* in 2012 examined the effect of the steroidal saponin extracted from *Dioscorea zingiberensis* on Human and murine carcinoma cells *in vitro* and indicated that steroidal saponin extracted suppressed cell proliferation in a dose and time dependent manner against cancer cells and was more effective on murine colon carcinoma cells C26 which their findings are in agreement to dependency of our results in time and concentration[25].

Beit-Yannai *et al.* in 2011 studied the inhibitory effect and mode of action of steroidal saponins (spirostane, furostane) from *Balanites aegyptiaca* against MCF-7 human breast cancer cells and HT-29 human colon cancer cells and were concluded spirostane showed significant anti proliferative activity against both cell lines while furostane revealed considerable selectivity of growth inhibition[26].

In summary, our data demonstrate presence of saponin in Persian Gulf brittle star (*O. erinaceus*) by convenient method and indicate that with comparation to above research reports saponin extracted (80% ethanol fraction) from *O. erinaceus* exhibited hemolytic and moderate cytotoxic activity on HeLa cancer cells *in vitro* which due to appropriate cytotoxicity will be useful for drug discovery investigation. Based on these findings, for clinical application saponin isolated from brittle star must be examined as anticancer chemopreventive drug against cervical cancer cells *in vivo*.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Saunders FR, Wallace HM. On the natural chemoprevention of cancer. *Plant Physiol Biochem* 2010; **48**(7): 621-626.

[2] Mayer AM, Rodríguez AD, Berlinck RG, Fusetani N. Marine pharmacology in 2007-8: marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous system, and other miscellaneous mechanisms of action. *Comp Biochem Physiol C Toxicol Pharmacol* 2011; **153**(2): 191-222.

[3] Wang WH, Hong JK, Lee CO, Cho HY, Shin S, Jung JH. Bioactive metabolite from the brittle star *Ophioplocus japonicus*. *Nat Prod Sci* 2004; **10**(6): 253-261.

[4] Andersson L, Bohlin L, Iorizzi M, Riccio R, Minale L, Moreno-López W. Biological activity of saponins and saponin-like compounds from starfish and brittle-stars. *Toxicon* 1989; **27**(2): 179-188.

[5] Prokofeva NG, Chaikina EL, Kicha AA, Ivanchina NV. Biological activities of steroid glycosides from starfish. *Comp Biochem Physiol Part B Biochem Mol Biol* 2003; **134**: 695-701.

[6] Thakur M, Melzig MF, Fuchs H, Weng A. Chemistry and pharmacology of saponins: special focus on cytotoxic properties. *Botanics: Targets and Therapy* 2011; **2011**: 19-29.

[7] Podolak I, Galanty A, Sobolewska D. Saponins as cytotoxic agents: a review. *Phytochem Rev* 2010; **9**: 425-474.

[8] Sun H, Chen L, Wang J, Wang K, Zhou J. Structure-function relationship of the saponins from the roots of *Platycodon grandiflorum* for hemolytic and adjuvant activity. *Int Immunopharmacol* 2011; **11**(12): 2047-2056.

[9] Lai L, Hsieh SC, Huang HY, Chou C. Effect of lactic fermentation on the total phenolic saponin and phytic acid contents as well as anti-colon cancer cell proliferation activity of soymilk. *J Biosci Bioeng* 2013; **115**(5): 552-556.

[10] Man S, Gao W, Zhang Y, Huang L, Liu C. Chemical study and medical application of saponins as anti-cancer agents. *Fitoterapia* 2010; **81**: 703-714.

[11] Yücekutlu AN, Bildacı I. determination of plant saponins and some of *Gypsophila* species: a review of the literature. *Hacettepe J Biol Chem* 2008; **36**(2): 129-135.

[12] Lacaille-Dubois MA, Wagner H. A review of the biological and pharmacological activities of saponins. *Phytomedicine* 1996; **2**(4): 363-386.

[13] Mats MN, Korkhov VV, Stepanov VR, Kupera EV, Oleĭnikova GK, Anisimov MM. [The contraceptive activity of triterpene glycosides the total sum of holotoxins A1 and B1 and holothurin A in an experiment]. *Farmakol Toksikol* 1990; **53**(2): 45-47. Russian.

[14] Dyck SV, Gerbaux P, Flammang P. Qualitative and quantitative saponin contents in five sea cucumbers from the Indian ocean. *Mar Drugs* 2010; **8**: 173-189.

[15] Hu XQ, Wang YM, Wang JF, Xue Y, Li ZJ, Nagao K, et al. Dietary saponins of sea cucumber alleviate orotic acid-induced fatty liver in rats via PPARalpha and SREBP-1c signaling. *Lipids Health Dis* 2010; **9**: 25.

[16] Oleszek W, Bialy Z. Chromatographic determination of plant saponins-an update (2002-2005). *J Chromatogr A* 2006; **1112**: 78-91.

[17] Fatemi SMR, Jamili S, Valinassab T, Kuranlu N. Diversity of Ophiuroidea from Lengeh Portand Qeshm island in the Persian Gulf. *J Fish Aquat Sci* 2010; **5**(1): 42-48.

[18] Keshavarz M, Mohammadikia D, Dabbagh AR. The echinoderms fauna in intertidal zone of southern oli village coast (Boushehr, Persian Gulf). *J Anim Sci Adv* 2012; **2**(5): 495-498.

[19] Kareru PG, Keriko JM, Gachanja AN, Kenji GM. Direct detection of triterpenoid saponin in medicinal plants. *Afr J Tradit Complement Altern Med* 2008; **5**(1): 56-60.

[20] Inalegwu B, Sodipo OA. Phytochemical screening and haemolytic activities of crude and purified saponins of aqueous and methanolic extracts of leaves of *Tephrosia vogelii* Hook. F. *Asian J Plant Sci Res* 2013; **3**(5): 7-11.

[21] Sun Y, Li M, Liu J. Haemolytic activities and adjuvant effect of *Anemone raddeana* saponins (ARS) on the immune responses to ovalbumin in mice. *Int Immunopharmacol* 2008; **8**: 1095-1102.

[22] Hassan SM, Haq AU, Byrd JA, Berhow MA, Cartwright AL, Bailey CA. Haemolytic and antimicrobial activities of saponin-rich extracts from guar meal. *Food Chem* 2010; **119**(2): 600-605.

[23] Sharma OP, Kumar N, Singh B, Bhat TK. An improved method for thin layer chromatographic analysis of saponins. *Food Chem* 2012; **132**(1): 671-674.

[24] Xu QM, Shu Z, He WJ, Chen LY, Yang SL, Yang G, et al. Antitumor activity of *Pulsatilla chinensis* (Bunge) Regel saponins in human liver tumor 7402 cells *in vitro* and *in vivo*. *Phytomedicine* 2012; **19**(3-4): 293-300.

[25] Tong QY, He Y, Zhao QB, Qing Y, Huang W, Wu XH. Cytotoxicity and apoptosis-inducing effect of steroidal saponins from *Dioscorea zingiberensis* Wright against cancer cells. *Steroids* 2012; **77**(12): 1219-1217.

[26] Beit-Yannai E, Ben-Shabat S, Goldschmidt N, Chapagain BP, Liu RH, Wiesman Z. Antiproliferative activity of steroidal saponins from *Balanites aegyptiaca*-an *in vitro* study. *Phytochem Lett* 2011; **4**(1): 43-47.