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Combination of TiO_2 nanoparticles and *Echinometra mathaeis* gonad extracts: *In vitro* and *in vivo* scolicidal activity against hydatid cysts

Azita Navvabi [a], Ahmad Homaei [d,*], Shahram Khademvatan [b,**], Mohammad Hassan Khadem Ansari [c], Mousa Keshavarz [a]

[a] *Department of Biology, Faculty of Marine Science and Technology, University of Hormozgan, Bandar Abbas, Iran*
[b] *Department of Medical Parasitology and Mycology and Cellular and Molecular Research Center, Urmia University of Medical Sciences, Urmia, Iran*
[c] *Department of Biochemistry, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran*
[d] *Department of Marine Biology, Faculty of Marine Science and Technology, University of Hormozgan, P.O. Box 3995, Bandar Abbas, Iran*

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ABSTRACT

Echinococcosis (hydatid cyst) is a zoonotic disease in sheep, cattle, pigs, rats, and humans that is caused by larvae of the tapeworm *Echinococcus granulosus*. Several studies have shown that invertebrates exhibit activity against various parasites, but no studies are available of effects of extracts of the sea urchin *Echinometra mathaei* on hydatid cysts. Here, *E. mathaeis* gonad extracts were investigated for their effects on hydatid cysts, in the absence and presence of TiO_2 nanoparticles. Different concentrations of *E. mathaeis* gonad extracts with and without TiO_2 were applied for different times and the scolicidal activity of each of these conditions was determined on viable protoscolices. 15 μg/ml gonad extract + TiO_2 was sufficient to kill 84% of the protoscolices after 60 min incubation. *In vivo* studies were performed on mice injected with *E. granulosus* protoscolices. Gonad extracts and gonad extracts combined with TiO_2 were given by oral gavage for a period of 3 months. Combined application of extract and TiO_2 increased the treatment efficiency, as shown by the reduction in number, size and volume of the cysts. Thus, sea urchin gonad extracts combined with TiO_2 nanoparticles have anti-hydatic and scolicidal effects.

1. Introduction

Cystic echinococcosis or hydatidosis is a neglected zoonotic helminthic disease that is caused by the larval stage of the parasitic tapeworm *Echinococcus granulosus.* Hydatidosis is transmitted by ingestion of parasite eggs in contaminated food and water, upon which the parasite develops into the larval stage in the visceral organs, especially in the liver and lungs, where it forms hydatid cysts (Ahmed et al., 2011).

Hydatidosis occurs worldwide. Approximately 40–80% of human patients harbor a solitary cyst within a single organ, while 20–40% of the individuals have multiple cysts or multiple organs involved. Liver is the most commonly infected organ, occurring in 70% of the patients, followed by the lungs, which usually are infected in 20–25% of the patients. Other organs which can be infected, but with lower frequency, are brain, kidneys, muscles, skin, and heart. However, hydatidosis may occur in any other organ. Up to recently, hydatidosis treatment involved surgery combined with chemotherapy with albendazole or mebendazole before and after surgery (Adas et al., 2009; Wang et al., 2016). However, the chemotherapy is often associated with adverse side effects (Caglar et al., 2008; Smego and Sebanego, 2005). Therefore, because of their fewer side effects and cost effectiveness, combinations of natural and mineral products and extracts have been suggested for the treatment of the disease and killing the larval form of the parasite, also known as protoscolices (Rahimi-Esboei et al., 2016).

In this respect, treatment with nanoparticles may offer new opportunities. For example, inorganic silver nanoparticles damage the cell membrane of bacteria and retard their growth (Li et al., 2010). Silver nanoparticles have also been applied successfully against human breast cancer and tapeworm protoscolices (Lashkarizadeh et al., 2015; Macha et al., 2019). For cost-effectiveness the silver nanoparticles may be combined with other therapeutic agents, and, in particular, marine echinoderms have gathered interest (Macha et al., 2019). Several species of echinoderms have been reported to exhibit antimicrobial activity by producing steroidal glycosides, polyhydroxylated sterols, naphthoquinone pigments, and antimicrobial peptides (Abubakar et al., 2012; Iorizzi et al., 1995). Sea urchins are marine echinoderms that

* Corresponding author. Department of Marine Biology, Faculty of Marine Science and Technology, University of Hormozgan, Bandar Abbas, P.O. Box 3995, Iran.
** Corresponding author.
E-mail addresses: a.homaei@hormozgan.ac.ir, a.homaei@gmail.com (A. Homaei), khademvatan@yahoo.com (S. Khademvatan).

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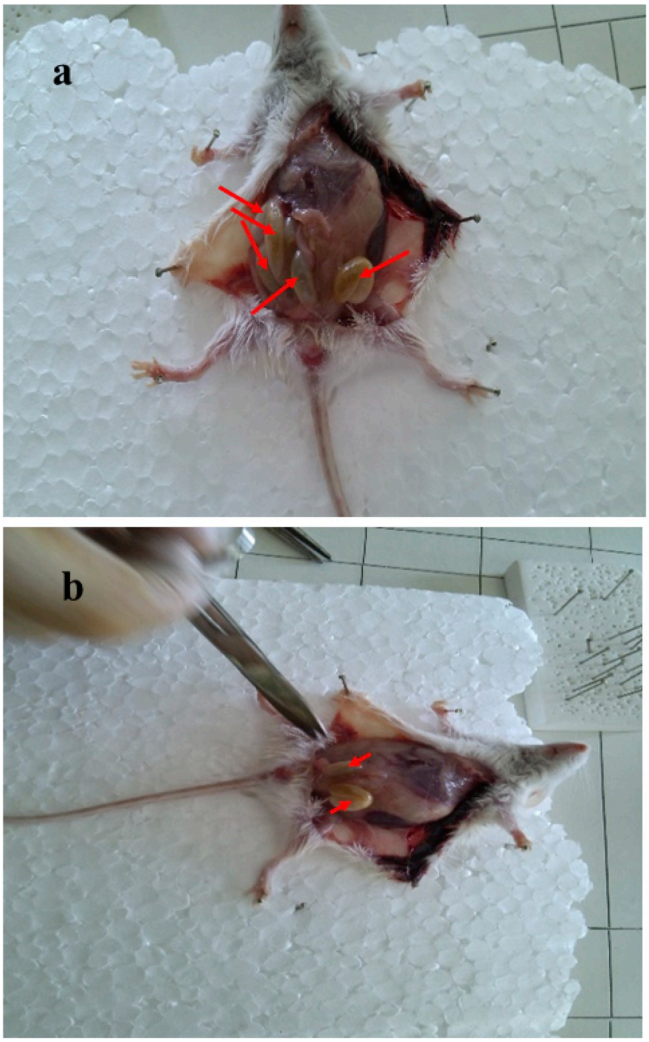

Fig. 1. Application of the combination of nanoparticles and sea urchin extracts showing the decrease in the number, size and volume of cysts before (a) and after the treatment (b).

produce secondary metabolites with drug-like properties (Sheean et al., 2007). Among others, a glycolipid derivative from a sea urchin intestinal extract was shown to possess significant anti-tumor activity (Sahara et al., 1997). Echinoderms have a poor physical defense system, and therefore, during evolution, they developed strong chemical defense strategies. Especially, the gonads which contain the reproductive cells, are well protected by a hard shell, and they contain echinochrome A, a polyhydroxylated naphthoquinone with strong antibacterial and antioxidant activity (Shankarlal et al., 2011). Indeed, Sheean et al. showed that a crude extract of *Heliocidaris erythrogramma* gonads displays antibacterial, antioxidant, anti-inflammatory, and anti-tumor and hepatocyte toxicity activity (Sheean et al., 2007).

Experimental infection of mice with protoscolices provides a well-established model for the study of the evolution of the disease and finding novel treatments for hydatid cysts. The objective of the present study is to find novel treatment options for hydatid cyst infections by evaluating whether sea urchin gonad extracts in combination with TiO_2 exhibit activity against alive protoscolices.

2. Materials and methods

Echinometra mathaei sea urchins were collected from the Persian Gulf at Hormozgan. After rinsing them with distilled water, and removing the spines, a gap was made in the peristomal membrane, the coelomic fluid was drained, and the gonads were separated, washed out, and kept in sterile condition and frozen. A fraction of them was separated, weighted, and homogenized in PBS for 20 min using an ultrasonic homogenizer.

TiO_2 nanoparticles were obtained from Sigma. Expected dose of TiO_2 mix with normal saline at 37 °C, then homogenized for 30 s.

2.1. Collection of hydatid samples and exposure

Protoscolices of *E. granulosus* were obtained aseptically from hydatid cysts present in livers of naturally infected sheep and goats slaughtered at Urmia, West Azerbaijan, and brought to the parasitology laboratory. The hydatid fluid was aspirated aseptically using a 20 ml syringe and left for 30 min for the protoscolices to settle down. The supernatant was discarded and the protoscolices were washed two times with PBS (pH 7.2) solution. To obtain the proportion of live protoscolices, the viability of the protoscolices was determined by their flame cell motility and dyeing with 0.1% eosin (Kayaalp et al., 2001). They were kept overnight in RPMI medium at 4 °C. Viable protoscolices were incubated *in vitro* with gonad extract, or with a combination of gonad extract and TiO_2, at different concentrations (1, 5, 10, 20 μg/ml and 1, 5, 15 μg/ml respectively). Incubation times were 5, 15, 30, and 60 min to attain total lethality. The tests were repeated three times. Gonad extracts had less scolicidal activity than when combined with TiO_2 nanoparticles. Caspase-3 activity was determined using the caspase-3 kit (Zell bio).

2.2. In vivo efficacy of gonad extraction combined with nanoparticles

BALB/c mice aged 4–5 weeks, with a body weight of 18–25 g, were infected by injection with 2000 viable protoscolices suspended in 0.5 ml phosphate buffer, pH 7, and randomly divided into four equal groups of 5 mice. Formation of hydatic cysts in mice occurs as early as after 4 months. Three parasitological parameters were considered when a cyst was found. These were the cyst size, the status of the cyst fluid, and the viability of the cysts.

Protoscolices from randomly selected mice were collected under aseptic conditions from hydatid cysts, and their viability was determined by their flame cell motility and dyeing with 0.1% eosin. Each group received the following treatment schemes for 3 months: group 1 was the negative control, group 2 was the positive control, group 3 received gonad extracts by oral gavage, group 4 was treated with gonad extracts combined with TiO_2, and group 5 received TiO_2 only. After the end of the 3-month treatment period, mice were dissected and the peritoneal cavity was opened for removing, counting, measuring, and histological analysis of the hydatid cysts. The hydatid cysts were drawn randomly from every group and studied for pathologic changes in the cyst. The size of the cysts was measured using a caliper. The study was in accordance with the ethics standards of "Helsinki Protocol of Laboratory Animal Care"'.

2.3. Statistical analysis

All tests were carried out in triplicate. Analysis of data was performed with the SPSS statistical package version 16. The data were presented as the mean ± SD. In this survey $p < 0.05$ was considered statistically significant.

Ethics approval

All animal experiments were performed according to the standards of the ethic committee board of the University of Hormozgan in full accordance with the World Medical Association Declaration of Helsinki.

3. Results

3.1. In vitro effects of gonad extracts on protoscolices

All treatments showed scolicidal effects, but the gonad extracts, when used separately, had less scolicidal activity than when combined with TiO_2 (Table 1). The viability of the protoscolices after 60 min exposure to 20 µg/ml gonad extract, or 15 µg/ml gonad extract with TiO_2 was 17.33%, or 15.30%, respectively (Table 2). The TiO_2 only treatment had less scolicidal activity at all concentrations than when combined with gonad extract, and the mortality rate was significantly increased when combined with gonad extract ($p < 0.05$) (Table 3). By increasing the exposure time, the mortality rate was significantly increased ($p < 0.05$).

3.2. Gonad extracts combined with TiO_2 affect the cysts of E. granulosus protoscolices in the mouse model

Hydatid cysts developed in 95% of the infected animals. 15 µg/ml gonad extracts in combination with TiO_2 is more effective in killing protoscolices than gonad extracts alone (Fig. 1). The treatment results are shown in Table 4. The number of hydatid cysts in the control group was 15–18. In group 3, which received 15 µg/ml gonad extracts combined with TiO_2 for 90 days, the cysts were of small size, or were completely gone. In the negative control group, a high number of cysts was observed. Based on these findings, we conclude that application of gonad extract combined with TiO_2 nanoparticles clearly reduces the number, size, and volume of the cysts. Table 4 shows the morphological changes after 3-months treatment of the mice. The size of the cysts in this group was about two times smaller than that of the cysts in the control group. In group 2, which received gonad extract only, the size of the cysts was larger than that of the cysts of group 3, which received the combination treatment, but smaller than the cysts of the control group. No adverse effects were observed in the drug treated groups: none of the mice exhibited any aberrant behavior during the treatment.

We tested whether concentrations of these combination extract which effectively *in vitro E. granulosus* protoscolices had high effect on the cysts of mice. Na_2HPO_4 extract of gonad extracts + TiO_2 recorded high activity at 15 µg/ml after 60 min treatment.

4. Discussion

Cystic echinococcosis is a worldwide-occurring zoonotic parasitic disease (Khademvatan et al., 2019). The common treatment consists of surgery of the tapeworm cyst combined with chemotherapy with drugs like albendazole and praziquantel (Reuter et al., 2010). Unfortunately, the treatment is not always successful, and suffers from undesirable side effects, such as neutropenia, elevation of liver enzyme levels, or alopecia (Dvorožňáková et al., 2004; Polat et al., 2005). Improvement of the therapy is thus urgently needed.

Table 1
Protoscolicidal effects of gonad extracts on protoscolices of hydatid cysts using different exposure times and extract concentrations.

Extract concentration (µg/ml)	5 min	Viability of protoscolices (%)	30 min	60 min
		15 min		
0	98.58 ± 0.01	98.38 ± 0.02	97.18 ± 0.02	95.18 ± 0.02
1	78.83 ± 0.20	62.69 ± 0.03	58.10 ± 0.10	41.93 ± 0.22
5	55.09 ± 0.14	50.05 ± 0.11	45 ± 0.02	39 ± 0.01
10	42.05 ± 0.22	40.07 ± 0.06	28.05 ± 0.05	24.0 ± 0.05
20	30.18 ± 0.22	25.39 ± 0.04	20.06 ± 0.05	17.33 ± 0.56

Table 2
Protoscolicidal effects of gonad extracts in combination of TiO_2 on the protoscolices of hydatid cysts using different exposure times and extract concentrations.

Extract concentration (µg/ml)	5 min	Viability of protoscolices (%)	30 min	60 min
		15 min		
0	98.52 ± 0.02	97.26 ± 0.02	95.32 ± 0.02	95.08 ± 0.07
1	74.97 ± 0.03	60.01 ± 0.09	52.02 ± 0.11	35 ± 0.10
5	46.82 ± 0.16	47.17 ± 0.16	34.76 ± 0.22	27.03 ± 0.06
15	32.30 ± 0.01	28.91 ± 0.10	19.85 ± 0.17	15.30 ± 0.15

Table 3
Scolicidal effects of TiO_2 extraction on protoscolices of hydatid cysts *in vitro*.

Extract concentration (µg/ml)	10 min	Viability of protoscolices (%)	30 min	60 min
		20 min		
2	89.08 ± 0.08	87.03 ± 0.05	78.19 ± 0.09	64.89 ± 0.11
5	79.15 ± 0.04	62.49 ± 0.06	56.98 ± 0.02	50.09 ± 0.07
10	58 ± 0.10	52.68 ± 0.02	47.24 ± 0.08	45.18 ± 0.17
20	51.99 ± 0.01	40.99 ± 0.01	39.68 ± 0.02	32.03 ± 0.05

Here, we investigated the combination of two different therapeutic approaches to combat cystic echinococcosis, using TiO_2 nanoparticles and/or organ extracts of marine invertebrates. Nanoparticles have attracted considerable attention as promising materials for biological and pharmaceutical applications because of their large surface-to-volume ratio and high penetration power (Khatua et al., 2019). In particular, cytotoxic nanoparticles have been successfully used as therapeutic agents against diverse forms of cancer, but they are also effective against pathogenic bacteria, viruses, fungi, and parasites (Balachandar et al., 2019; Kanagamani et al., 2019). In contrast, various invertebrates have developed effective chemical defense strategies to fight micro-organisms and higher predators (Ribeiro et al., 2010). Amongst others, they produce compounds with antioxidant and anti-microbial activity, as well as compounds that act against some viruses (Bodnar, 2013). In particular, sea urchin gonadal tissue contains bioactive metabolites with anti-inflammatory and anti-tumor activity, which may be useful for the treatment of human diseases (Sheean et al., 2007).

In the present study, the anti-parasitic properties of sea urchin gonad extracts were investigated *in vitro* and *in vivo*, and in the absence and presence of TiO_2 nanoparticles. Our experiments showed that, *in vitro*, the combination of gonad extracts and TiO_2 had higher scolicidal activity than gonad extracts on their own. Especially, at a concentration of 15 µg/ml, 84.7% of the protoscolices were killed after 60 min of exposure. Adding TiO_2 nanoparticles to the gonad extracts increased the mortality rate significantly. Moreover, *in vivo* treatment of mice infected with hydatid cysts was also effective, as evident from the lower number, smaller size and volume of the cysts compared with the control group. Again, addition of TiO_2 nanoparticles improved the efficacy of the treatment significantly.

Titanium dioxide has frequently been used as a nanomaterial because of its non-toxicity, stable physicochemical properties, and low cost. Several studies have reported the antimicrobial activity of TiO_2 and its synergism when used with other compounds (ChiChen, 2017). For instance, TiO_2 nanoparticles positively influenced the antimicrobial and

Table 4
Mean *E. granulosus* cyst size and mean volume in mice.

Groups	Cyst size)mm (	Mean cyst size (mm)	Cyst volume	Mean cyst volume	Percentage infection
Negative control	0	0	0	0	0
Positive control	5–11.2	8.53	0.1–0.8	8.53	100 (5/5mice)
Gonad extract	3.3–7	5.33	0.1–0.5	5.25	100 (5/5mice)
Gonad extract + TiO_2	1–4	2	0.1–0.3	5.20	100 (5/5mice)
TiO_2 only	4–8	6	0.1–0.6	5.56	100 (5/5mice)

disinfectant efficacy of silver nanoparticles against three fungi, *Aspergillus niger*, *Penicillium spinulosum,* and *Stachybotrys chartarum*, and two bacteria, *Escherichia coli* and *Staphylococcus epidermidis* (ChiChen, 2017). Here, we have shown that TiO_2 is also effective in improving the treatment of cystic echinococcosis using sea urchin gonad extracts. Further studies are necessary to establish the safety and effectiveness of the therapy in humans.

Declaration of competing interest

We declare no conflict of interest in submission of this manuscript.

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References

Abubakar, L., Mwangi, C., Uku, J., Ndirangu, S., 2012. Antimicrobial Activity of Various Extracts of the Sea Urchin Tripneustes Gratilla (Echinoidea). Missing Bibliographic Details.

Adas, G., Arikan, S., Kemik, O., Oner, A., Sahip, N., Karatepe, O., 2009. Use of albendazole sulfoxide, albendazole sulfone, and combined solutions as scolicidal agents on hydatid cysts (in vitro study). World J. Gastroenterol. 15 (1), 112 (Please include last page).

Ahmed, M.A., Sabeeh, H., Amal, K.H., 2011. Anti protoscolices activity of nonadecoic acid, phithalic acid, diflorophenyl undecylester and 1,2-benzendic ester extracted from Cladophora Crispata and Hapaloshihon Aureus ESTER. Thi-Qar Medical Journal 5 (2), 69–81 (Check title).

Balachandar, R., Gurumoorthy, P., Karmegam, N., Barabadi, H., Subbaiya, R., Anand, K., et al., 2019. Plant-mediated synthesis, characterization and bactericidal potential of emerging silver nanoparticles using stem extract of Phyllanthus pinnatus: a recent advance in phytonanotechnology. J. Clust. Sci. 30 (6), 1481–1488.

Bodnar, A., 2013. Proteomic profiles reveal age-related changes in coelomic fluid of sea urchin species with different life spans. Exp. Gerontol. 48 (5), 525–530.

Caglar, R., Yuzbasioglu, M.F., Bulbuloglu, E., Gul, M., Ezberci, F., Kale, I.T., 2008. In vitro effectiveness of different chemical agents on scolices of hydatid cyst. J. Investig. Surg. 21 (2), 71–75.

Chen, Y.C., Yu, K.P., 2017. Enhanced antimicrobial efficacy of thermal-reduced silver nanoparticles supported by titanium dioxide. Colloids Surfaces B Biointerfaces 154, 195–202.

Dvorožňáková, E., Hrčková, G., Borošková, Z., Velebný, S., Dubinský, P., 2004. Effect of treatment with free and liposomized albendazole on selected immunological parameters and cyst growth in mice infected with Echinococcus multilocularis. Parasitol. Int. 53 (4), 315–325.

Iorizzi, M., Bryan, P., McClintock, J., Minale, L., Palagiano, E., Maurelli, S., Zollo, F., 1995. Chemical and biological investigation of the polar constituents of the starfish Luidia clathrata, collected in the Gulf of Mexico. J. Nat. Prod. 58 (5), 653–671.

Kanagamani, K., Muthukrishnan, P., Shankar, K., Kathiresan, A., Barabadi, H., Saravanan, M., 2019. Antimicrobial, cytotoxicity and photocatalytic degradation of norfloxacin using kleinia grandiflora mediated silver nanoparticles. J. Clust. Sci. 1–10.

Kayaalp, C., Balkan, M., Aydin, C., Ozgurtas, T., Tanyuksel, M., Kirimlioglu, Pekcan, M., 2001. Hypertonic saline in hydatid disease. World J. Surg. 25 (8), 975–979.

Khademvatan, S., Majidiani, H., Foroutan, M., Tappeh, K.H., Khalkhali, H.R., 2019. Echinococcus granulosus genotypes in Iran: a systematic review. J. Helminthol. 93 (2), 131–138.

Khatua, A., Priyadarshini, E., Rajamani, P., Patel, A., Kumar, J., Naik, A., Meena, R., 2019. Phytosynthesis, characterization and fungicidal potential of emerging gold nanoparticles using Pongamia pinnata leave extract: a novel approach in nanoparticle synthesis. J. Clust. Sci. 1–7.

Lashkarizadeh, M.R., Asgaripour, K., Dezaki, E.S., Harandi, M.F., 2015. Comparison of scolicidal effects of amphotricin B, silver nanoparticles, _and Foeniculum vulgare Mill on hydatid cysts protoscoleces. Iran. J. Parasitol. 10 (2), 206.

Li, W.R., Xie, X.B., Shi, Q.S., Zeng, H.Y., You-Sheng, O.Y., Chen, Y.B., 2010. Antibacterial activity and mechanism of silver nanoparticles on Escherichia coli. Appl. Microbiol. Biotechnol. 85 (4), 1115–1122.

Macha, I.J., Ben-Nissan, B., Müller, W.H., Cazalbou, S., 2019. Marine nanopharmaceuticals for drug delivery and targeting. In: Marine-derived Biomaterials for Tissue Engineering Applications. Springer, Singapore, pp. 207–221.

Polat, C., Dervisoglu, A., Hokelek, M., Yetim, I., Buyukkarabacak, Y., Ozkutuk, Y., Erzurumlu, K., 2005. Dual treatment of albendazole in hepatic hydatidosis: new therapeutic modality in 52 cases. J. Gastroenterol. Hepatol. 20 (3), 421–425.

Rahimi-Esboei, B., Ebrahimzadeh, M.A., Fathi, H., Rezaei Anzahaei, F., 2016. Scolicidal effect of Allium sativum flowers on hydatid cyst protoscolices. Eur. Rev. Med. Pharmacol. Sci. 20 (1), 129–132.

Reuter, S., Beisler, T., Kern, P., 2010. Combined albendazole and amphotericin B against Echinococcus multilocularis in vitro. Acta Trop. 115 (3), 270–274.

Ribeiro, S.M., Bianco, É.M., Rogers, R., Teixeira, V.L., Pereira, R.C., 2010. Chemical defense of Hymeniacidon heliophila (Porifera: halichondrida) against tropical predators. Braz. J. Oceanogr. 58 (4), 315–321.

Sahara, H., Ishikawa, M., Takahashi, N., Ohtani, S., Sato, N., Gasa, Kikuchi, K., 1997. In vivo anti-tumour effect of 3'-sulphonoquinovosyl 1'-monoacylglyceride isolated from sea urchin (Strongylocentrotus intermedius) intestine. Br. J. Canc. 75 (3), 324.

Shankarlal, S., Prabu, K., Natarajan, E., 2011. Antimicrobial and antioxidant activity of purple sea urchin shell (Salmacis virgulata L. Agassiz and Desor 1846). Am.-Eurasian J. Sci. Res. 6, 178–181.

Sheean, P.D., Hodges, L.D., Kalafatis, N., Wright, P.F., Wynne, P.M., Whitehouse, M.W., Macrides, T.A., 2007. Bioactivity of extracts from gonadal tissue of the edible Australian purple sea urchin Heliocidaris erythrogramma. J. Sci. Food Agric. 87 (4), 694–701.

Smego Jr., R.A., Sebanego, P., 2005. Treatment options for hepatic cystic echinococcosis. Int. J. Infect. Dis. 9 (2), 69–76.

Wang, S., Yang, T., Zhang, X., Xia, J., Guo, J., Wang, X., Wu, X., 2016. Construction of in vivo fluorescent imaging of Echinococcus granulosus in a mouse model. Korean J. Parasitol. 54 (3), 291.