Extraction and identification of bioactive compounds from starfish using Gas Chromatograph-Mass Spectropohotometry

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Abstract : Starfish have been the research topic in many pharmacological and chemical pharmacological laboratories due to their complex secondary metabolites and types of bioactivities. The starfishes were shown to contain the bioactive compounds which exhibit various activities including antiinflammatory, cytotoxicity, hemolytic, antiviral and antibacterial effects. Starfish are also considered as an extremely rich source of biological active compounds, including saponin. However, very few studies were performed to analyze the biological properties of starfish and the bioactive compounds. In this study an attempt was made to analyze the bioactive compounds from the solvent extract of star fish. The compounds such as, N-Allyl-N,N-dimethylamine, 9-Octadecenoic acid (Z)-, 2, 3-dihydroxypropyl ester, 3-Octadecene, (E) –, 1-Hexanamine, N-ethyl-, 5-Tetradecanol acetate, 1H-Indole, 5-methyl-2-phenyl-, Pyridjpyrimidine, 4-phenyl- Pyridjpyrimidine, 4-phenyl-, 2-Ethylacridine, Docosanoic acid, Benzene, 2-[(tert-butyldimethylsilyl) oxy]-1-isopropyl-4-methyl- and Cyclotrisiloxane, hexamethyl- were identified. These compounds may have potent activity against various diseases.

Keywords: Star fish, solvent extract, bioactive compounds

I. INTRODUCTION

Starfish, also called sea stars are invertebrates belonging to the class Asteroidea from the phylum Echinodermata. Except a few species that inhabit brackishwaters environment, starfish are benthic organisms found in marine environments. Starfish are mainly considered as the most abundant source of polyhydroxy steroids. Though the distribution of polyhydroxy steroids has high significance among marine species in general, polyhydroxy steroids with similar structures are found in most starfish species (Wang, 2003). Moreover, the industrial applications of the secondary metabolites of starfish have received much more attention throughout the world. Steroidal glycosides, which are composed of a polyhydroxylated steroidal aglycone and a carbohydrate portion containing only one or two monosaccharide units, are a growing sub group of the active glycoside compounds that have been isolated from starfishes (Iorizzi et al., 1986).

Extracts and saponins isolated from starfish show a broad spectrum of biological effects: cytotoxic, hemolytic, antifungal and antiviral activities (Verbist 1993). Although a high number of sulphated steroidal glycosides from starfish have been characterized in the past few decades (Auria et al., 1993), only a few studies concerning the biological activities of pure compounds have been studied. Starfish can be divided into three structural classes: sulphated steroidal glycosides (asterosaponins), steroidal cyclic glycosides and polyhydroxylated steroidal glycosides (Minale et al., 1986). Chemically related polyhydroxylated sterols as well as sulphated polyhydroxylated sterols and disulfated sterols have been recently isolated (d'Auria et al., 1984). Extracts and pure saponins from starfish have a broad spectrum of biological activity.

Sea stars are benthic free living echinoderm has evolved with rich sources of bioactive metabolites such as steroidal glycosides, steroids, alkaloids, glycolipids and phospholipids (de Marino et al., 1997). Especially steroidal glycosides and related compounds are predominant metabolites in sea stars and have a broad variety of various biological activities such as cytotoxic, repellent, antineoplastic, hemolytic, ichthyotoxic, antifungal,antimicrobial, antiviral and anti-inflammatory (Andersson et al., 1989). Imbricatine from the sea star *Dermasterias imbricata* is the first benzyltetrahydroisoquinolone alkaloid from a non-plant source and shows in the NCI human cell line screen (Carte, 1996). Cerebrosides and gangliosides have been reported to exhibit many pharmacological effects including neuritogenic and antitumour activities (Emdo et al., 1986). Saponins are widespread in sea stars (Iorizzi et al., 2001).

Antioxidant activities of solvent and aqueous extracts and isolated marine natural products from sea stars were also established. In a study, Suguna et al. (2014) reported potential antioxidant activity of the sea star *L. maculata*. The study also further established the presence of steroidal glycosides and glucocerebrosides which might be the responsible agents for the antioxidant activity. In another study, antioxidant activities and neuroprotective effect were reported from the polysaccharides extracted from the starfish *Asterias rollestoni* (Zhanga et al., 2013). It was also found out that the mannoglucan sulfate had the highest antioxidant activity among all polysaccharides tested. These useful information on bioactivities of extracts from different marine organisms were mostly conducted in temperate and polar regions, thus implying the need to further and advance interest on marine organisms from biodiversity-rich tropical regions (Llacuna et al., 2016). In the present study an attempt was made to identify bioactive compounds from star fish using GC-MS analysis.

II. MATERIALS AND METHODS

Extractions of metabolites from star fish

For the extraction of crude compounds, about 500 gm of dried starfishes was initially kept in two litre of methanol, followed by 90% and 60% ethanol for 5 days each. The solvents were then removed after squeezing of specimen and filtered through Whatman No. 1 filter paper. The solvents were evaporated further at very low pressure by using a rotary evaporator at 35°C. The resultant extract was finally dried in vacuum desiccators and stored at 4°C for further study.

Gas chromatography-mass spectroscopy GC-MS) analysis of crude extract from star fish

The GC-MS analysis of crude extract from star fish was carried out using Thermo GC-trace standard non-polar column (Dimension: 30 meters, film: 0.25 µM). The injector temperature was set at 260 °C during the chromatographic run. About 1 µl of sample was injected into the instrument. The oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min^{-1}; and 300 °C, where it was held for 6 min. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 50-650 (m/z). The ionization voltage was 70 eV. In this chromatography hydrogen was used as the carrier gas. This column (30 m × 0.25 mm) was closely fitted to a Perkin Elmer gas chromatography which was equipped with a flame ionization detector. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

III. Results and Discussion

In the present study crude extract from starch fish showed various bioactive compounds. The compounds such as, N-Allyl-N,N-dimethylamine, 9-Octadecenoic acid (Z)-, 2, 3-dihydroxypropyl ester, 3-Octadecene, (E) –, 1-Hexanamine, N-ethyl-, 5-Tetradecanol acetate, 1H-Indole, 5-methyl-2-phenyl-, Pyridjpyrimidine, 4-phenyl- Pyridjpyrimidine, 4-phenyl-, 2-Ethylacridine, Docosanoic acid, Benzene, 2-[(tert-butyldimethylsilyl) oxy]-1-isopropyl-4-methyl- and Cyclotrisiloxane, hexamethyl- were identified (Fig. 1). These compounds may have potent activity against various diseases. The GC-MS spectrum of identified compounds were shown in Fig. 2-13. Marine invertebrates have been found to produce a good diversity of various bioactive secondary metabolites and be a potential source for novel drug discovery. Several drug discovery projects have screened echinoderms for antibiotic activities (Levina et al., 2008). Palagiano et al. (1996) isolated 20 steroid glycosides from the starfish *Henricia downeyae* that causes growth inhibition in fungi and bacteria. Aminin et al. (1995) first identified disulfated polyhydroxysteroids that turned out to be potent Ca^{2+}agonists in mammalian cell systems from star fish (Aminin et al., 1995). The chemical composition of three echinoderm extracts, namely *A. irregularis*, *L. sarsi* and *O. albida*, was evaluated by GC–MS which is considered as a fast and simple method for metabolite profiling of extracts from marine organisms (Pereira et al., 2012). Moreover, previous studies on the chemical composition of other echinoderms such as *Austrocedrus chilensis* and *Diadema setosum* revealed that it has high proportions of oleic acid and glycerol 1-palmitate, respectively (Minn et al., 2004)) which play important roles in membrane integrity and stability (Motsinger-Reif et al., 2013).

Previous studies are supports the activity of sea stars extracts obtained from various geographical locations and many drug discovery projects have screened echinoderms against various human fungi, bacterial, viral and fish pathogens (Sri Kumaran et al., 2011). Devi *et al.* (2011) reported that the body wall extract of sea star *Pentaceraster affinis* showed considerable activity against *Shigella flexineri*, *Acinetobacter* sp., and moderate activity against *Sreptococcus pyogenes*. They also suggested that the compounds inhibited the activity of pathogen may be saponins and saponin like steroid substances. James (2010) reported that considerable toxicity is found in the echinoderms and the “crown of thorns” *Acanthaster planci* is highly toxic to human as well as fish predators. Sri Kumaran *et al.* (2011) also reported that the butanol extracts of *Protoreaster lincki* showed high activity against *S. paratyphi* and *K. pneumonia*. Jha and Xu Zi-rong (2004) has reported various bioactive compounds from sea stars, sea urchin and sea cucumbers and reported that the major compounds in the sea stars are saponins, steroidal glycosidesand alkaloids.

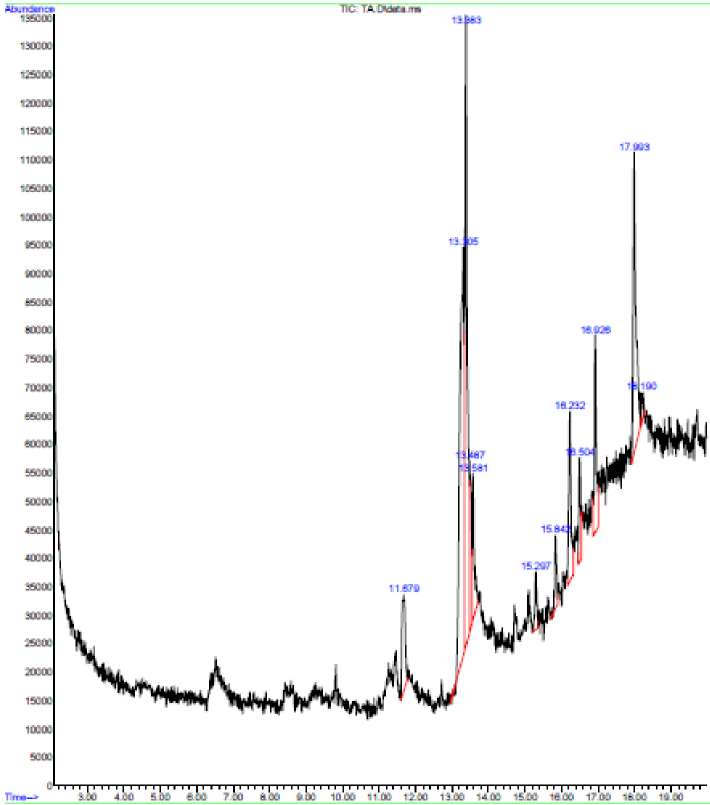

Fig. 1. Gas Chromatography – Mass Spectrophotometry profile of secondary metabolites from starfish.

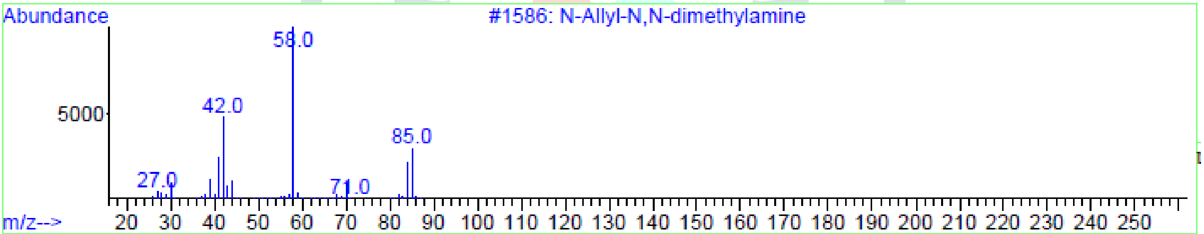

Fig. 2. GC – MS spectrum of N-Allyl-N,N-dimethylamine from Starfish.

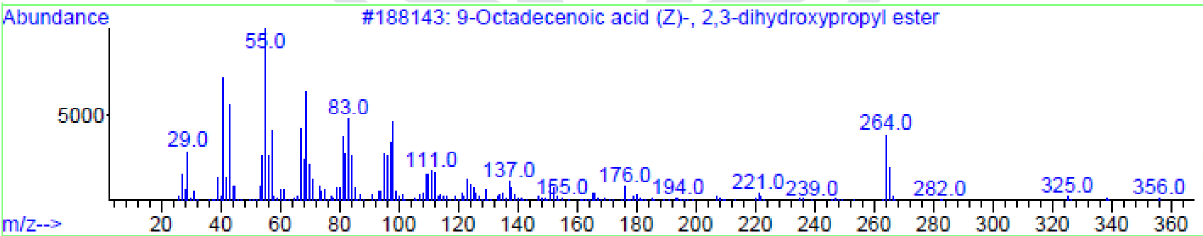

Fig. 3. GC – MS spectrum of 9-Octadecenoic acid (Z)-, 2, 3-dihydroxypropyl ester from Starfish.

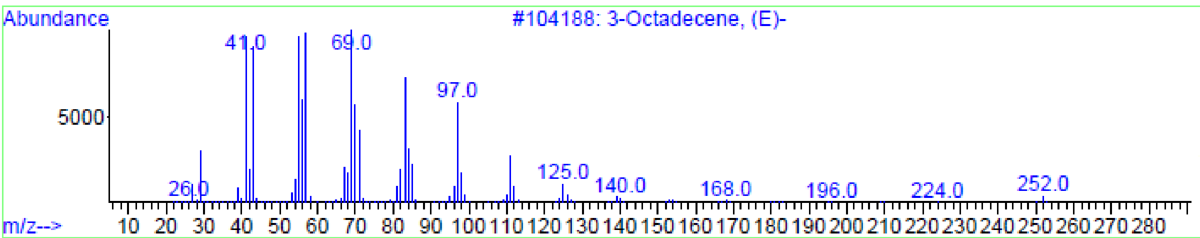

Fig. 4. GC – MS spectrum of 3-Octadecene, (E) - from Starfish.

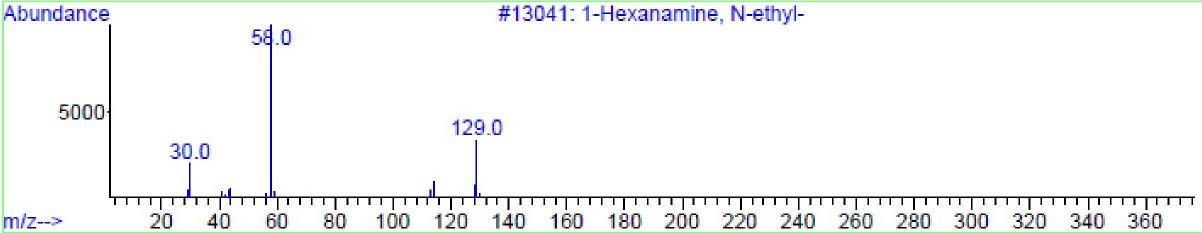

Fig. 5. GC – MS spectrum of 1-Hexanamine, N-ethyl- from Starfish.

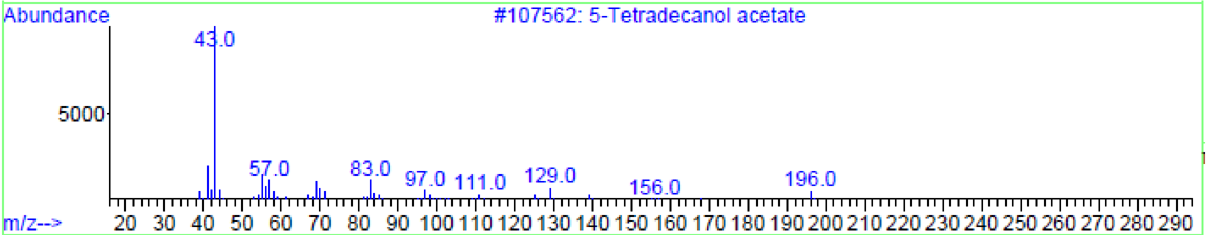

Fig. 6. GC – MS spectrum of 5-Tetradecanol acetate from Starfish.

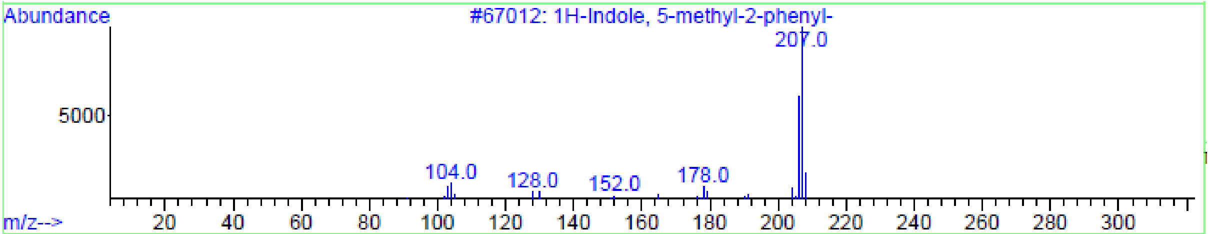

Fig. 7. GC – MS spectrum of 1H-Indole, 5-methyl-2-phenyl- from Starfish.

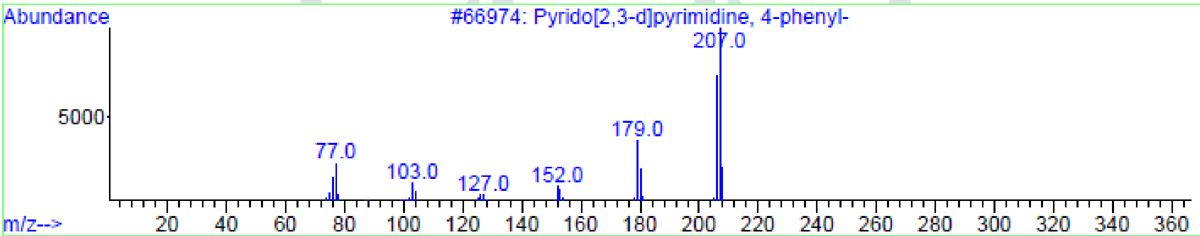

Fig. 8. GC – MS spectrum of Pyridjpyrimidine, 4-phenyl- from Starfish.

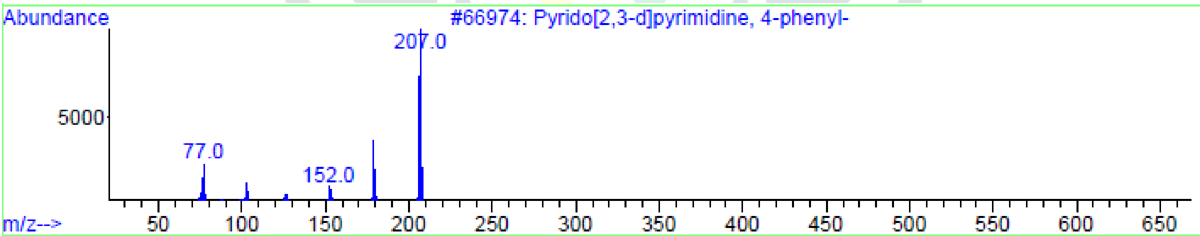

Fig. 9. GC – MS spectrum of Pyridjpyrimidine, 4-phenyl- from Starfish.

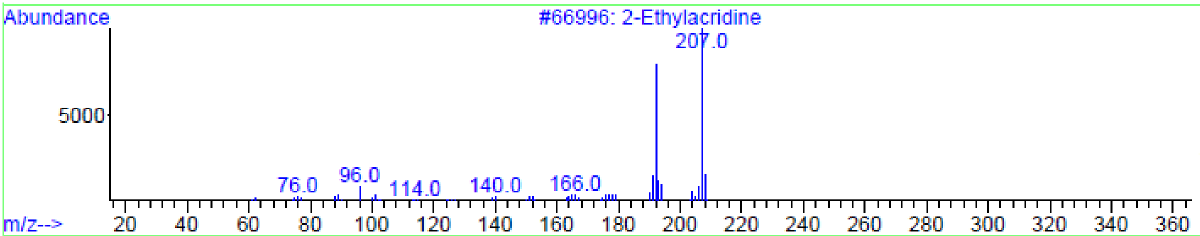

Fig. 10. GC – MS spectrum of 2-Ethylacridine from Starfish.

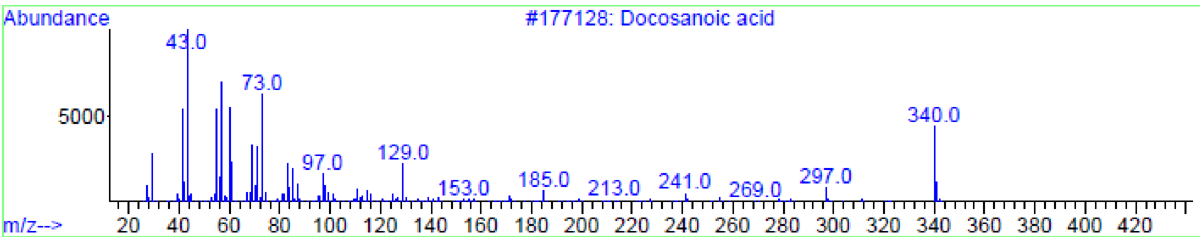

Fig. 11. GC – MS spectrum of Docosanoic acid from Starfish.

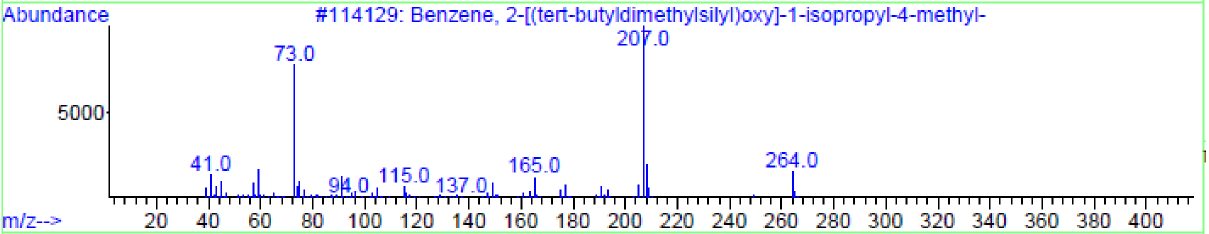

Fig. 12. GC – MS spectrum of Benzene, 2-[(tert-butyldimethylsilyl)oxy]-1-isopropyl-4-methyl- from Starfish.

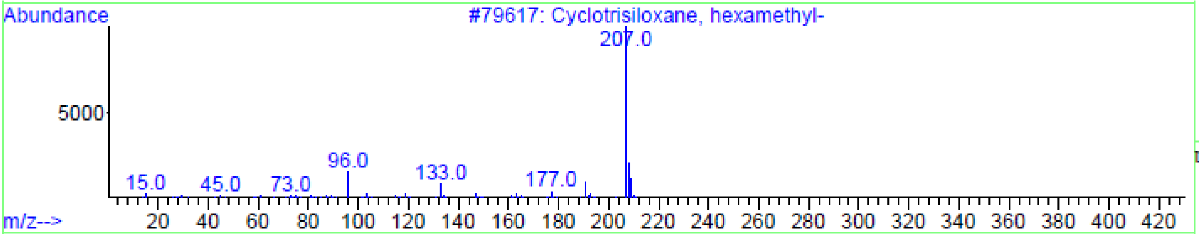

Fig. 13. GC – MS spectrum of Cyclotrisiloxane, hexamethyl- from Starfish.

The secondary metabolites of starfish include a remarkable diversity of various steroids, including sterols, polyhydroxysteroids, mono- and biosides of polyhydroxysteroid, and steroid oligoglycosides called asterosaponins (Kicha et al., 2001). There are various reports of the anti-inflammatory and anticancer activities of bioactive compounds obtained from starfish, such as the anti-inflammatory activity of starfish steroids (Thao et al., 2013), and anticancer activity of polyhydroxylated steroids (Levina et al., 2010), cerebroside (Zuo et al., 2013), and polysaccharides on human breast cancer cell lines (Lee et al., 2011) and colorectal adenocarcinoma cell lies (Lee et al., 2012). Cerebrocides purified from *A. pectinifera* starfish were also cytotoxic against two human cancer cells in a dose-dependent and time-dependent manner up to 400 µg/mL concentrations (Zuo et al., 2013). A polysaccharide of *A. pectinifera* starfish also inhibited the growth of human breast (Lee et al., 2011) and colorectal (Lee et al., 2012) cancer cells. Therefore, steroids, spingolipids or polysaccharides obtained from starfish might act as anticancer compounds. In a study, starfish saponin was extracted with different solvents such as methanol and *n*-butanol. It is very interesting to collect steroidal glycosides from *n*-butanol extracts of entire animals of starfish (Kicha et al., 2011). The starfish was chopped in small pieces and homogenized with methanol. The extract was performed with *n*-butanol and then water, to afford sulfated steroidal glycosides (asterosaponins) from starfish (Kicha et al., 2011). These glycosides are also studied in the research of chemical constituents and biological activities of starfish, with considerable clinical interest, since they show several physiological, pharmacological, and immunological activities (Dong et al., 2011).

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