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Original Article

# Pharmacological and chemical properties of some marine echinoderms

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ABSTRACT

Echinoderms have attracted the attention of scientists over the past few years after identifying a variety of unique structures endowed by interesting biological properties. However, the Moroccan coast biodiversity is still uninvestigated. In our ongoing attempts to valorize the rich Moroccan marine environment, this study aimed at assessing the antimicrobial activity of extracts obtained from three echinoderms *Astropecten irregularis*, *Luidia sarsi* and *Ophiura albida* against the human pathogens: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica* and *Bacillus subtilis*. Moreover, their antioxidant activities were tested using standard methods in addition to the antidiabetic activity which has been evaluated *in vitro* against α-amylase and α-glucosidase enzymes. HPLC-DAD-QTOF-MS analysis revealed a significant content of some phenolic compounds such as pyrogallol, gallic, sinapic, ferulic, *p*-hydroxybenzoic and salicylic acids whose existence can be related to the endophytic fungi and/or dietary intake whereas GC-MS analysis exhibited diverse chemical structures such as cholesterol, oleic acid and glycerol 1-palmitate.



## Introduction

The branch of echinoderms makes up one of the essential and distinctive components of the marine animal kingdom containing more than 7000 living species and 13 000 extinct (Brusca and Brusca, 2003). Echinoderms are classified into five classes: *Crinoidea*, *Holothuroidea*, *Echinoidea*, *Asteroidea* and *Ophiuroidea* (Brusca and Brusca, 2003) which are deeply distinguished from other classes by their particular anatomy and ecology. Echinoderm species occupy a variety of subtidal substrates ranging from rough gravel to fine sludge, despite they are more commonly found in sand in the coastal waters (Freeman et al., 1998). In traditional medicine, echinoderms such as *Oreaster reticulates*, *Echinaster echinophorus*, *Luidia senegalensis*, *Mellita quinquiesperforata* and *Echinometra lucunter* are used in Brazil against asthma, alcoholism, bronchitis, diabetes and heart diseases. In addition, *Echinaster brasiliensis* is drank as a tea from the powdered toasted starfish (Costa-Neto, 1999). This therapeutic potential is due to the rich chemical diversity of echinoderms as an interesting source in ethnopharmacology and natural products research (Uzair et al., 2011; Gupta and Abu-Ghannam, 2011). Metabolites from echinoderms could be subdivided into steroids, glycosides, ceramide derivatives and miscellaneous compounds. Recent investigations resulted in the isolation of many compounds from echinoderms species, such as triterpene glycosides, glycosaminoglycans, chondroitin sulphate and neuritogenic gangliosides (Kelly, 2005; Higuchi et al., 2007). Recent studies have reported that phytoplankton accumulates phenolic compounds in a carbonic environment, leading to an increase in their levels in marine organisms which have profound consequences on marine ecosystem and seafood quality with the possibility that fishery industries could be influenced as a result of progressive ocean changes (Jin

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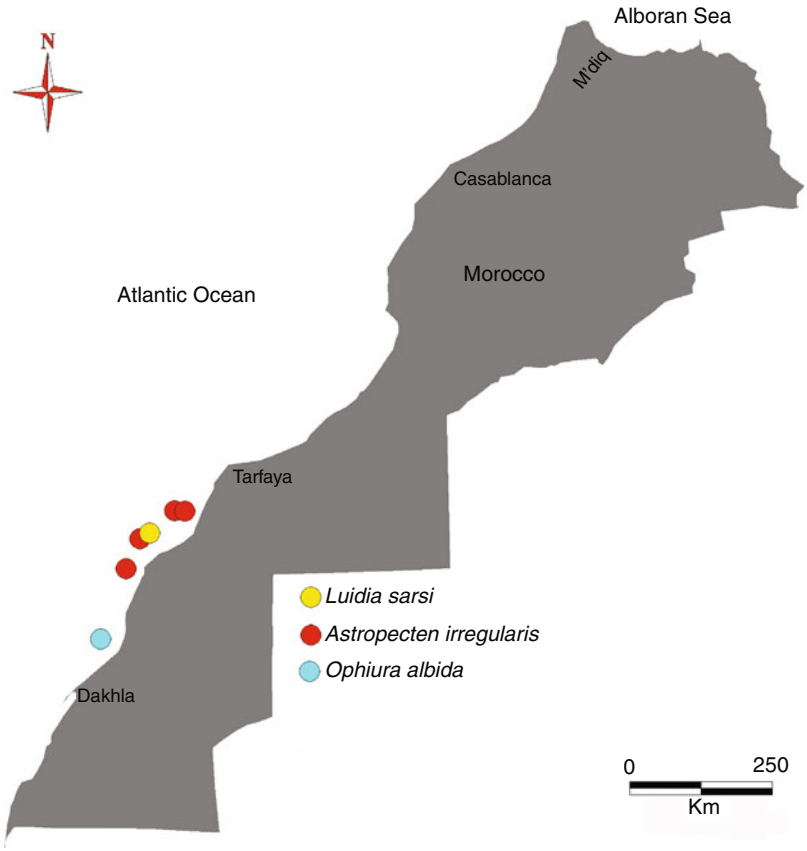


**Figure 1.** Location of collection sites.

**Table 1**
Coordinates of collection sites.

| | | *A. irregularis* | *L. sarsi* | *O. albida* |
|---|---|---|---|---|
| 15°10′35″N | 24°38′45″ | – | – | x |
| 14°36′7″N | 25°57′9″ | x | – | – |
| 14°13′8″N | 26°31′6″ | x | – | – |
| 13°37′28″N | 26°58′32″ | x | x | – |
| 13°29′66″N | 26°58′85″ | x | – | – |

et al., 2015). Despite the interesting contribution of many reports (Sasaki et al., 1985; Haug et al., 2002), research directed toward chemistry and bioactivity of echinoderm compounds is still in its infancy, regarding the huge contribution of marine products in modern therapeutic development. Data from literature reported the antibacterial property of the body wall, coelomocytes and eggs in a variety of echinoderm species (Stabili and Pagliara, 1994; Stabili et al., 1996; Haug et al., 2002). To the best of our knowledge, the antidiabetic effects of such organism are still not or little investigated.

Currently, there is an increased interest in new chemical and therapeutic agents modulating postprandial rise in blood glucose levels and therefore considered as key agents in the management of diabetes and its complications. For instance, α-glucosidase is a key enzyme in the intestinal glucose metabolism, localized in the epithelium of the small intestine and catalysing the cleavage of disaccharides to glucose. It can be considered as one of the main targets in antidiabetic therapy (Toeller, 1994; Henrissat, 1998; Kimura, 2000).

In order to evaluate the chemical diversity and bioactivities of Moroccan echinoderms, three species namely *Astropecten irregularis* (Pennant, 1777), *Luidia sarsi* Düben & Koren, 1846 and *Ophiura albida* Forbes, 1839 were investigated. In this study, we assessed the potential antidiabetic effect *via* α-amylase and α-glucosidase inhibition. The antioxidant activity linked to many pathological diseases has also been investigated using different methods. In addition, the antimicrobial activities of the collected echinoderms from Moroccan coast were evaluated against many pathogenic isolates.

## Material and methods

### *Echinoderms collection, identification and extraction*

Specimens of *Astropecten irregularis* (Pennant, 1777), *Luidia sarsi* Düben & Koren, and *Ophiura albida* Forbes, 1839 were collected in May 2015 between 26 and 84 m of depths using rectangular dredge. The dredge was towed by the boat. All specimens were collected between Tarfaya and Dakhla (Table 1 and Fig. 1). Species identification was carried out by Dr. O. Wangensteen (University of Barcelona) and N. Tamsouri (Institut National de Recherche Halieutique, INRH, Morocco). Voucher specimens were deposited at the institute under the following numbers LPNA15, LPNA16, LPNA17. The fresh animals (25 g each) were cut into small pieces and extracted three times with MeOH. MeOH extracts were filtered, concentrated and stored for evaluation.

### *Phenolic, flavonoid and tannin contents*

The amount of phenolic contents was determined according to Folin-Ciocalteu method as described in our previous works (Marmouzi et al., 2015). The total phenolic content was measured as milligrams of gallic acid equivalents per gram of extract dry weight (mg GAE/g edw). The total flavonoids in the extracts were determined using a colorimetric assay method. The flavonoid

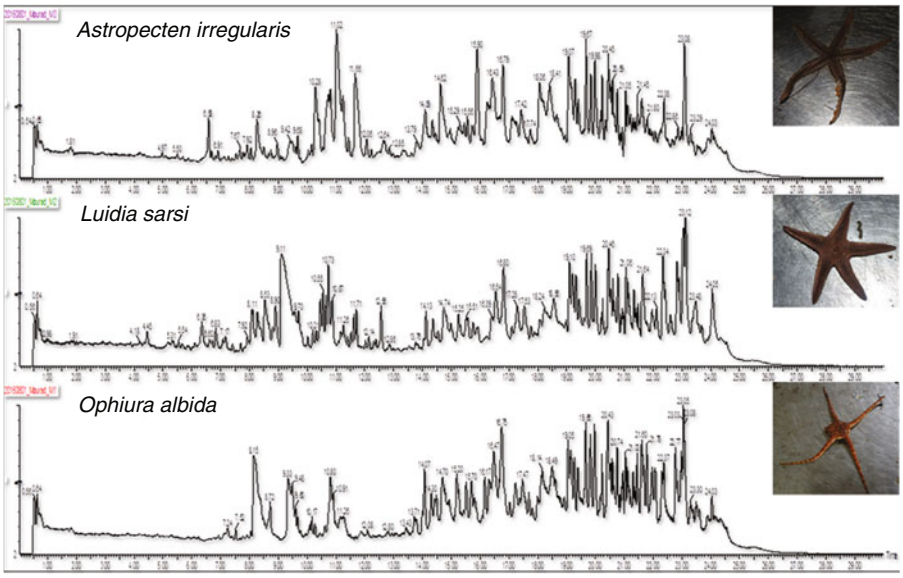


**Figure 2.** Chromatograms and photography of echinoderm species.

content was determined as rutin equivalent from the calibration curve of rutin standard solution (mg RE/g edw). For the condensed tannin content measurements, the results were expressed in catechin equivalent (mg CE/g edw).

### *HPLC-DAD-QTOF-MS analysis*

HPLC-DAD-QTOF-MS system consisted of a binary pump (G1312 A; Agilent 1100) and an autosampler (G1330B) coupled to a mass spectrometer equipped with an electrospray ionizer source (MS; ESI; Micromass Quattro Micro; Waters, Milford, MA, USA). Reversed-phase HPLC separation was carried out using Zorbax C18 column (100 mm × 2.1 mm × 1.7 μm). The mass spectrometer was operated in negative mode with the following parameters: capillary voltage, 3.0 kV; cone voltage, 20 V; and extractor, 2 V. Source temperature was 100 °C, desolvation temperature was 350 °C, cone gas flow was 30 l/h, and desolvation gas flow was 350 l/h. The mobile-phase components were 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The mobile-phase gradient was: 0 min, 90% A; 0–18 min, 30% A; 18–20 min, 30% A; 20–23 min, 30% A; 23–25 min, 90% A; 25–30 min, 90% A. The injection volume was 10 μl and the column temperature was 35 °C. The flow rate of the mobile phase was 0.500 ml/min. The phenolic acids, flavanols and flavonols were identified on the basis of their retention times, MS spectra and molecular-ion identification.

### *GC-MS analysis*

Gas chromatography combined with mass spectrometry was used for the identification of the main compounds. The analysis was performed on a GC-MS Clarus 600/560DMS Perkin-Elmer (Bridgeport, USA), equipped with an automatic injector. The system is controlled by a mass Turbo Software (Windows XP SP2). The stationary phase is a column Supelco® (L 30 m × 0.25 ID × DF 0.25) Elite-5MS phase, the carrier gas is helium at a flow rate of 0.8 ml/min. The oven was programmed from 75 to 320 °C at a gradient of 10 °C per min with a pre-heating of the transfer line at 325 and 250 °C at the source. Ionization is caused by electron impact (EI). Identification of the chemical components in extracts was based on computer matching with NIST and Wiley 275 libraries.

### *Antioxidant activities*

The free radical scavenging activity of the echinoderm extracts was measured by 2,2′-diphenyl-1-picrylhydrazyl hydrate (DPPH) (Blois, 1958; Marmouzi et al., 2016). The ferric ions ($Fe^{3+}$) reducing antioxidant power (FRAP) method (Oyaizu, 1986) was used to measure the reducing capacity of the extracts with a slight modification, which involves the presence of extracts to reduce the ferricyanide complex to the ferrous form. The reducing power of the extracts was represented as ascorbic acid equivalent (mg AAE/g edw). The antioxidant activities samples are expressed as TEAC values (Re et al., 1999), defined as the concentration of standard Trolox with the same antioxidant capacity of the extract under investigation (mg TE/g edw).

### *α-Amylase inhibition*

The α-amylase inhibition assay was conducted according to the previously described protocol (Marmouzi et al., 2017; Sayah et al., 2017). Briefly, 250 μl of the sample was mixed with 250 μl of α-amylase (240 U/ml, in 0.02 M phosphate buffer, pH 6.9, with 0.006 M NaCl). After incubating at 37 °C for 10 min, 250 μl of 1% (w/v) soluble starch (in 0.02 M phosphate buffer, pH 6.9) was added and the mixture was further incubated at 37 °C for 30 min followed by adding 500 μl of DNS color reagent. Afterwards, the reaction was stopped by heating in a boiling water bath for 10 min and then cooling to room temperature. The mixture has been diluted with 2 ml of buffer and the absorbance was measured at 540 nm.

The inhibition percentage (%) was calculated by the following equation for both enzymatic assays:

$$\text{Inhibition}\,(\%) = \frac{(\text{AC} - \text{ACb}) - (\text{AS} - \text{ASb})}{\text{AC} - \text{ACb}}$$

where AC is the absorbance of the control, ACb the control blank, AS the absorbance of the sample and ASb the absorbance of the sample blank.

### *α-Glucosidase inhibition*

As described previously (Marmouzi et al., 2017), the α-glucosidase enzyme (0.1 U/ml) and substrate *p*-nitrophenyl-α-D-glucopyranoside (*p*-NPG, 1 mM) were dissolved in potassium

**Table 2**
GC-MS analysis of Echinoderms extracts.

| No. | Rt | Elemental composition | Compound name | *m/z* | Extracts |
|---|---|---|---|---|---|
| 1 | 8.81 | $C_8H_{16}O_6$ | Ethyl D-glucopyranoside | 208.20 | AI |
| 2 | 10.79 | $C_{11}H_{18}N_2O_2$ | Pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl) | 210.27 | AI |
| 3 | 12.42 | $C_{18}H_{34}O_2$ | Oleic acid | 282.46 | AI, LS |
| 4 | 14.29 | $C_{19}H_{38}O_4$ | Glycerol 1-palmitate | 330.50 | AI |
| 5 | 14.38 | $C_{21}H_{34}O_2$ | 5,8,11,14-Eicosatetraenoic acid, methyl ester | 318.49 | AI |
| 6 | 17.41 | $C_{28}H_{25}NO_7$ | 3′,8,8′-Trimethoxy-3-piperidyl-2,2′-binaphthalene-1,1′,4,4′-tetrone | 487.50 | AI |
| 7 | 20.40 | $C_{28}H_{46}O_4$ | 1,2-Benzenedicarboxylic acid, bis(8-methylnonyl)ester | 446.66 | LS |
| 8 | 21.86 | $C_{37}H_{76}O$ | 1-Heptatriacontanol | 536.99 | AI |
| 9 | 24.07 | $C_{27}H_{46}O$ | Cholesterol | 386.65 | AI, LS, OA |

phosphate buffer (0.1 M, pH 6.7) and all samples were dissolved in distilled water. The inhibitor (150 μl) was pre-incubated with the enzyme (100 μl) at 37 °C for 10 min and then the substrate (200 μl) was added to the reaction mixture. The enzymatic reaction was performed at 37 °C for 30 min. The reaction was then terminated by the addition 1 ml of 1 M $Na_2CO_3$. All samples were analyzed in triplicate with different concentrations to determine the $IC_{50}$ values and the absorbance was recorded at 405 nm.

### *Antimicrobial activity*

The antimicrobial activity was carried out against five bacterial strains from the Institut Pasteur collection (CIP): *Staphylococcus aureus* CIP 483, *Bacillus subtilis* CIP 5262, *Escherichia coli* CIP 53126, *Pseudomonas aeruginosa* CIP 82118 and *Salmonella enterica* CIP 8039. For the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the extracts, a modified resazurin microtiter-plate assay was used as reported by Sarker et al. (2007). Under aseptic conditions, 96-well microtiter plates were labeled. The first row of microtiter plate was filled with 100 μl of test materials at a concentration of 25 mg/ml. To all other wells of the microtiter plates, we added 100 μl of tryptic soya broth. A serial dilution was achieved by starting transferring 100 μl test material from first row to the subsequent wells in the next row of the same column and so that each well has 100 μl of test material in serially descending concentrations. Then, 10 μl was taken from bacterial suspension ($10^8$ CFU/ml) and added to each well and the microplates were placed in an incubator set at 37 °C for 24 h. Each plate had a set of controls: a column with an antibiotic as positive control [chloramphenicol in a serial dilution (25 mg/ml)], a column with all solutions except the test material and a column with all solutions with the exception of the bacterial suspension. Finally, the resazurin solution was prepared by dissolving 270 mg of resazurin powder in 40 ml sterile distilled water. The solution was mixed on a vortex mixer until the powder was completely dissolved and the solution was homogenous (McNicholl et al., 2006). A volume of 10 μl of resazurin solution as indicator was added in each well after incubation. The microplates were placed again in an incubator at 37 °C for just 2 h. The color change was then assessed visually. Any color changes from purple to pink or colorless were recorded as positive. The lowest concentrations at which color change occurred were taken as MIC values.

The MBC is the lowest concentration of the substance that leaves at most 0.01% of surviving germs. It is determined using a series of steps, undertaken after a minimum inhibitory concentration test has been completed (Ponce et al., 2003). Using a loop, we make streaks from the tubes in which no disorder was observed on plates containing TSA starting with the tube of the MIC. Following overnight incubation, we examined the MBC plates for colony growth or lack of growth for each dilution subcultured. No growth indicates that our crude extract was bactericidal at that dilution. Growth indicates that it was bacteriostatic but not bactericidal at that dilution.

### *Statistical analysis*

Data were expressed as the mean ± standard deviation (SD) for each measurement. The data were also analyzed by one-way analysis of variance (one-way ANOVA). *Post hoc* procedure was used for significance of difference ($p < 0.05$). Analysis was performed with Graph pad prism 6.

## Results and discussion

### *Chemical composition*

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). Identification of several compounds was achieved by identifying the peaks, using retention times ($R_t$) and mass spectra (MS). The obtained results from GC–MS chromatograms are reported in Table 2. It was observed that, ethyl D-glucopyranoside, pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl), oleic acid, glycerol 1-palmitate, 5,8,11,14-eicosatetraenoic acid, methyl ester, 3′,8,8′-trimethoxy-3-piperidyl-2,2′-binaphthalene-1,1′,4,4′-tetrone, 1-heptatriacontanol and cholesterol were detected in *A. irregularis* extracts, while only oleic acid, 1,2-benzenedicarboxylic acid, bis(8-methylnonyl) ester and cholesterol were detected in *L. sarsi* extracts. Oleic acid belongs to the omega-9 family and has the capacity to reduce the incidence of cardiovascular diseases as well as antidiabetic and anti-inflammatory properties. Regarding *O. albida*, none of the previously cited compounds was detected except cholesterol which is usually present in marine organisms including echinoderms as it constitute a bioactive chemical defense compound against critical environmental conditions (Minn et al., 2004). 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; Careaga et al., 2013) which play important roles in membrane integrity and stability (Motsinger-Reif et al., 2013).

Many toxic compounds can be accumulated in echinoderms from their endophytic and/or nutritive environment (Shim et al., 2005) such as tributyltin and triphenyltin accumulated from dietary uptake and from surrounding water. On the other hand, the endophytic environment of echinoderms can produce many compounds of medical interest. Phytoplanktons that constitute an important dietary source for sea stars and ophiura have been shown to overproduce phenolic compounds subsequently ingested by echinoderms (Jin et al., 2015). It is clearly noticed that echinoderm extracts contain much more than compounds from their own biosynthetic pathways.

HPLC-DAD-QTOF-MS analysis of MeOH extracts from echinoderm species allowed identifying six phenolic acids, by means of comparison based on retention times and mass fragmentation

**Table 3**
HPLC-DAD-QTOF-MS quantification of phenolic compounds (mg/kg).

| Phenolic acids | Rt | MW | *AI* | *LS* | *OA* |
|---|---|---|---|---|---|
| Pyrogallol | 0.59 | 126 | 3.02 ± 0.32 | 1.63 ± 0.12 | nd |
| Gallic acid | 0.76 | 170 | 0.21 ± 0.07 | 0.16 ± 0.04 | 0.06 ± 0.01 |
| *p*-Hydroxybenzoic acid | 1.68 | 138 | 5.49 ± 0.29 | 4.19 ± 0.21 | 4.49 ± 0.14 |
| Ferulic acid | 2.96 | 194 | 4.18 ± 0.18 | 4.78 ± 0.26 | nd* |
| Sinapic acid | 3.44 | 224 | nd | nd | 0.78 ± 0.07 |
| Salicylic acid | 4.42 | 138 | 0.12 ± 0.03 | 1.42 ± 0.17 | 0.20 ± 0.03 |

* nd (not detected).

**Table 4**
Phenolic contents and antioxidant activities of Echinoderm extracts.

| | *A. irregularis* | *L. sarsi* | *O. albida* |
|---|---|---|---|
| PC (mg GAE/g edw) | 110.71 ± 12.11 | 121.27 ± 15.32 | 113.85 ± 8.53 |
| FC (mg RE/g edw) | 35.10 ± 0.23 | 95.30 ± 12.45 | 65.20 ± 2.12 |
| CTC (mg CE/g edw) | 25.89 ± 3.21 | 10.94 ± 1.33 | 7.76 ± 0.91 |
| DPPH (mg TE/g edw) | 38.49 ± 2.45 | 3.20 ± 0.34 | 28.53 ± 1.39 |
| ABTS (mg TE/g edw) | 104.01 ± 6.90 | 112.20 ± 3.78 | 72.12 ± 7.78 |
| FRAP (mg AAE/g edw) | 17.58 ± 2.13 | 16.44 ± 1.43 | 34.40 ± 2.57 |

pattern with data obtained from reference standards (Fig. 2). The concentrations of each compounds calculated from calibration curves are reported in Table 3. Significant amounts were registered in each species: *A. irregularis* was much rich in pyrogallol (3.02 ± 0.32 mg/kg), gallic acid (0.21 ± 0.07 mg/kg) and *p*-hydroxybenzoic acid (5.49 ± 0.29 mg/kg). While *L. sarsi* contained relatively higher contents of ferulic (4.78 ± 0.26 mg/kg) and salicylic acids (1.42 ± 0.17 mg/kg). Sinapic acid has been recorded only in *O. albida* (0.78 ± 0.07 mg/kg).

*Antioxidant activity*

Antioxidant activity of different marine organisms has recently attracted much attention. In fact, antioxidants can protect the human body from free radicals and reactive oxygen species effects. They hinder the progress of many chronic diseases as well as lipid peroxidation. Many studies have reported that echinoderms showed marked antioxidant abilities (Mamelona et al., 2010; Esmat et al., 2013; Pachaiyappan et al., 2014). Different assays such as PC, FC, CTC, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, ABTS (azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radical cation scavenging effect and FRAP (ferric-reducing antioxidant power) were used to evaluate the antioxidant capacity of the echinoderms organic extracts. The obtained values of three different extracts (*A. irregularis*, *L. sarsi* and *O. albida*) are illustrated in Table 4. It is noteworthy to mention that significant differences in the distributions of FC and CTC were observed among the three different echinoderms extracts. Though phenolic compounds did not show significant differences, these phenolic compounds are known to be able to terminate free radicals, chelate metal ions that may catalyze the formation of reactive oxygen species (ROS) and promote lipid peroxidation (Archana et al., 2016). Regarding the capacity of a compound to scavenge DPPH radicals which depends directly on their capability of pairing the unpaired radical electron (Park et al., 2004), the highest DPPH radical scavenger (38.49 mg TE/g edw) was found in *A. irregularis* (28.53 mg TE/g edw) followed by *O. albida* (3.20 mg TE/g edw), whereas the lowest was present in *L. sarsi*. For ABTS, results revealed that *A. irregularis* possesses the greatest activity (104.01 mg TE/g edw) compared to *L. sarsi* 112.20 mg TE/g edw) and *O. albida* (72.12 mg TE/g edw). Concerning the FRAP assay that measures the capability of a compound to reduce a ferric oxidant ($Fe^{3+}$) into a ferrous complex ($Fe^{2+}$) by means of electron transfer (O'Sullivan et al., 2011). Results revealed a reducing capacity for three extracts and therefore their antioxidant activities. *O. albida* exhibited the highest activity at 43.4 mg AAE/g edw, while *A. irregularis* and *L. sarsi* displayed a lower activity with only 17.58 and 16.44 AAE/g edw, respectively.

**Table 5**
Antidiabetic activity of Echinoderm extracts (IC50 in μg/ml).

| | α-Amylase | α-Glucosidase |
|---|---|---|
| *A. irregularis* | 147.08 ± 4.32 | 539.97 ± 10.12 |
| *L. sarsi* | 150.52 ± 5.87 | 442.76 ± 9.87 |
| *O. albida* | 737.32 ± 8.43 | 872.29 ± 11.32 |
| Acarbose | 396.42 ± 5.16 | 199.53 ± 1.12 |

*Antidiabetic effect*

Antidiabetic activity of three different extracts (*A. irregularis*, *L. sarsi* and *O. albida*) was evaluated using acarbose as a standard antidiabetic drug that acts by inhibiting α-amylase and α-glucosidase activities (Oboh et al., 2016). α-Amylase and α-glucosidase are critical enzymes involved in the breakdown of α-glycosidic bonds of complex carbohydrates and intestinal absorption, respectively. Therefore, their inhibitors could retard the use of dietary carbohydrates, suppressing postprandial hyperglycaemia linked to type 2 diabetes (Lordan et al., 2013). It was observed that, the tested echinoderm extracts revealed an interesting antidiabetic activity since they exhibit significant inhibition of α-amylase (Fig. 3a) and α-glucosidase (Fig. 3b) in a concentration-dependent approach. In fact, Table 5 shows that regarding α-amylase, the $IC_{50}$ values for *A. irregularis* and *L. sarsi* extracts were 147.08 and 150.52 μg/ml, respectively, indicating their high effectiveness in comparison to acarbose, which showed an $IC_{50}$ of 396.42 μg/ml. The three tested echinoderm extracts displayed also inhibitory activities toward α-glucosidase with an $IC_{50}$ range of 442.76–872.29 μg/ml, proving its low activity compared to that 199.53 μg/ml for acarbose (Table 5). Thus, it can be deduced that the tested extracts have a higher inhibitory effect toward α-amylase than acarbose, while they are less effective toward α-glucosidase.

*Antimicrobial activity*

Echinoderms are considered as benthic organisms that can protect themselves against microbial infections and represent a rich and untapped resource for discovery of antibacterial compounds (Haug et al., 2002). They have stronger antibacterial effects than Porifera, Mollusca, Bryozoa or Annelida (Ridzwan et al., 1995). Previous studies reported antimicrobial activities from various species of Echinoderms (Kiani et al., 2014; Adibpour et al., 2014; Wang et al., 2014; Jebasingh et al., 2015). In this study, three echinoderm extracts were tested against five species of bacteria and the antibacterial activity was found in all of them (Table 6). The results show that *A. irregularis, L. sarsi* and *O. albida* extracts have antibacterial activities against *S. aureus* CIP 483, *E. coli* CIP 53126 and *B. subtilus* CIP 5262 at minimal inhibitory concentrations (MIC) of 12.50, 6.25 and 3.12 mg/ml, respectively. Additionally, the extracts from *A. irregularis* and *L. sarsi* proved to be active against *S. enterica* CIP

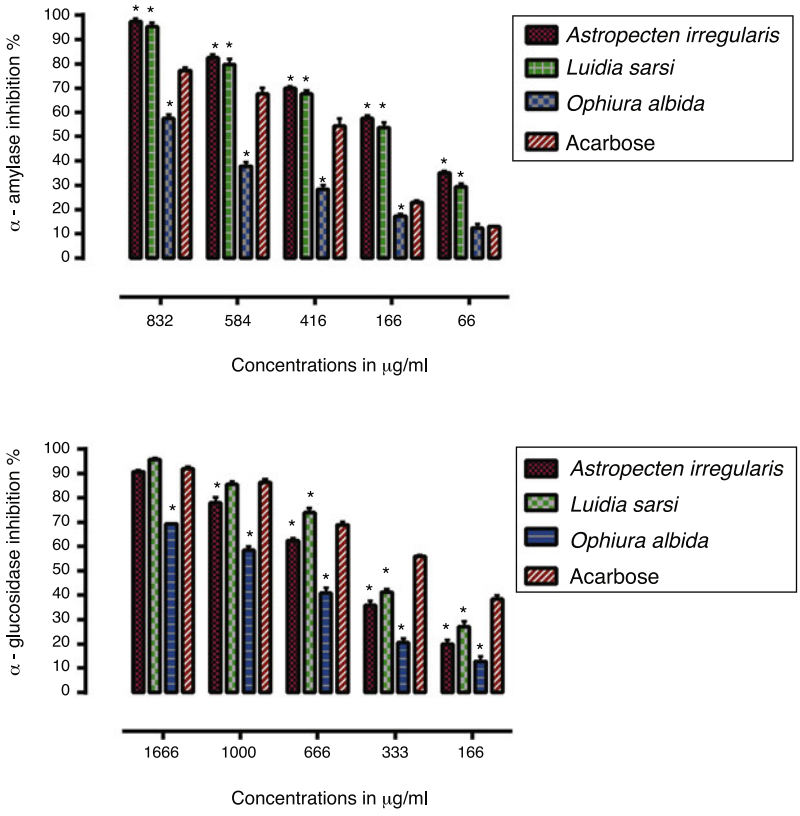


**Figure 3.** α-Amylase and α-glucosidase inhibition assays. Statistical differences are indicated (*) in comparison to acarbose.

**Table 6**
Antibacterial activity of Echinoderm extracts (MIC in mg/ml).

| | *AI* | *LS* | *OA* | Chloromphenicol |
|---|---|---|---|---|
| *E. coli* CIP 53126 | 6.25 | 6.25 | 6.25 | 0.095 |
| *S. aureus* CIP 483 | 12.50 | 12.50 | 12.50 | 0.05 |
| *S. enteric* CIP 8039 | 6.25 | 6.25 | 3.12 | 0.05 |
| *B. subtilus* CIP 5262 | 3.12 | 3.12 | 3.12 | 0.05 |
| *P. aeruginosa* CIP 82118 | 3.12 | 6.25 | 3.12 | 0.095 |

8039 at MIC value of 6.25 mg/ml, whereas *O. albida* revealed similar activity at MIC of 3.12 mg/ml. On the other hand, the extracts obtained from *A. irregularis* and *O. albida* displayed antibacterial effects on *P. aeruginosa* CIP 82118 at 3.12 mg/ml and at 6.25 mg/ml for *L. sarsi* extract.

Other species of echinoderms have been formerly tested for antibacterial properties with varied outcome. Stabili et al. (1994) have reported the antibacterial properties of the seminal plasma of *Paracentrotus lividus* against *Vibrio alginolyticus*. Adibpour et al. (2014) tested extracts from *Holothuria leucospilota* and found that they possess antibacterial effects against some selected species such as *P. aeruginosa, S. typhi, S. aureus and E. coli*. Jebasingh et al. (2015) reported that *Capillaster multiradiatus* extract displayed potential antibacterial compounds to hinder the human and fish pathogenic bacterial strains. These indicate that with advanced and in-depth research in this field, echinoderms extracts could be served as alternatives for antibiotic against pathogenic microorganism.

## Conclusions

Results of the present investigation demonstrated that *A. irregularis*, *L. sarsi* and *O. albida* possess promising antioxidant and antimicrobial activities, probably due to their accumulated phenolic compounds. We also found that they could significantly inhibit α-amylase and α-glucosidase involved in the digestion of starch and glucose production. These echinoderm extracts are prolific sources of nutritive compounds. They could have potential applications for use in pharmaceutical and food industries. In addition, they may serve as alternative antibiotic against pathogenic bacterial strains as well.

## Ethical disclosures

**Protection of human and animal subjects.** The authors declare that no experiments were performed on humans or animals for this study.

**Confidentiality of data.** The authors declare that they have followed the protocols of their work center on the publication of patient data.

**Right to privacy and informed consent.** The authors declare that no patient data appear in this article.

## Author's contributions

IM conceived, designed, performed and participated in all of the experiments; AA wrote the paper; NT collected and identified the species; MEH performed antimicrobial assays; MEJ analyzed the data and participated in antioxidant and antidiabetic activities; MK and RA participated in chemical analysis; SSE, YC and MEAF supervised the work and corrected the manuscript.

## Conflicts of interest

The authors declare no conflicts of interest.

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