RESEARCH ARTICLE Indian Journal of Animal Research, Volume 57 Issue 8: 1051-1058 (August 2023)

The Sea Urchin *Sphaerechinus granularis* (Lamarck, 1816) from the Mediterranean Sea: A New Natural Source of Antibacterial and Antioxidant Molecules

S. Amri[1,2], L. Benhalima[1,2], S. Belhaoues[1], A. Saoudi[1], M. Bensouilah[1]

10.18805/IJAR.BF-1485

ABSTRACT

Background: The marine organisms are well known to produce many bioactives molecules, which serve various functions. In the present paper, the sea urchin *Sphaerechinus granularis* was used as biological material for exploring the new bioactive molecules.

Methods: The study was carried out in spring 2019 (April), sea urchins *Sphaerechinus granularis* were harvested from coastal areas of El-Kala. All the sea urchins collected were transported to the laboratory in sea water. The test were opened and gonads removed. The gonads were pooled for biochemical and microbiological analysis.

Result: The screening revealed the presence of saponosides, mucilages, alkaloids, combined anthraquinones (C-heterosides), polyphenols and flavonoids. The biochemical analysis of the gonads has shown that they are rich in secondary metabolites. The antioxidant capacity indicated that the methanolic extract is better compared to the aqueous extract. The antibacterial effect were observed in the methanolic extract while the aqueous extract showed no antibacterial activity. The calculation of the ratio minimum inhibitory concentration/minimum bactericidal concentration had indicated a bactericidal effect with respect to Gram negative bacteria and bacteriostatic with respect to Gram positive bacteria for the methanolic extract. Aqueous extract reported no effect.

Key words: Antibacterial, Antioxidant, Screening, *Sphaerechinus granularis*.

INTRODUCTION

Antibiotics have a crucial role in fighting against many infectious diseases. However, with the increasing and often unwarranted use of these molecules, bacteria may become resistant to antibiotics (Benhalima *et al.,* 2015). The high level of antibiotic resistance might be due to the widespread and indiscriminate usage of antibiotics in the treatment (Sunder *et al.,* 2021), so it is needful to search for alternative compounds that could effectively inhibit these bacteria (Aksoy, 2021). The bioactive molecules extracted from marine organisms are an important new resource for obtaining useful compounds (Chen and Hwang, 2014), marine organisms are well known to produce many numerous bioactive molecules, which serve various functions, such as antibacterial, anticoagulant, anti-inflammatory and antitumor activities (Mayer *et al.,* 2011). Previous studies have proven that specific bioactive components that were extracted from sea urchins exhibit many types of activity (Li *et al.,* 2010 ; Schillaci *et al.,* 2010; Mamelona *et al.,* 2011). The antimicrobial activity in echinoderms has been reported in *Salmacis virgulata* (Shankarlal *et al.,* 2011), *Echinometra mathaei* (Kazemi *et al.,* 2016), *Diadema Setosum* (Sidiqi *et al.,* 2019) and *Paracentrotus lividus* (Chiaramonte *et al.,* 2021). The gonads are rich in valuable bioactive compounds, in addition, they can serve as a functional food to fight against inflammatory diseases, diabetes (Pozharitskaya *et al.,* 2015), tiredness (Shang *et al.,* 2018), antibacterial (Li *et al.,* 2015) and antiviral (Salas-Rojas *et al.,* 2014). The objective of the work is to search for new bioactive molecules from *Sphaerechinus granularis* sea urchins to fight against bacteria. For this we have, i) Investigate the consultants screening by testing for the presence of different classes of metabolites including alkaloids, anthocyanins, anthraquinones, flavonoids, leuco-anthocyanins, mucilages, phenols, reducing compound, saponins, steroids, tannins and triterpenoids. ii) Estimating the dosage of secondary metabolities (carotenoids, vitamin C and E, polyphenols and flavonoids). iii) Determination of antioxidant capacity by the DPPH radical scavenging assay and ferric reducing/antioxidant power assay. iiii) Perform antibacterial susceptibility assays through the antibacterial activity and also to determine the minimum inhibitory and bactericidal concentration.

[1]Laboratory of Ecobiology of Marine Environment and Coastlines, Faculty of Science, Badji Mokhtar University, BP12 Annaba 23000, Algeria.

[2]Faculty of Natural Sciences, Life Sciences, Earth and the Universe, 8 Mai 1945 University, BP401 Guelma 24000, Algeria.

Corresponding Author: S. Amri, Faculty of Natural Sciences, Life Sciences, Earth and the Universe, 8 Mai 1945 University, BP401 Guelma 24000, Algeria. Email: amri.sandra@univ-guelma.dz.

How to cite this article: Amri, S., Benhalima, L., Belhaoues, S., Saoudi, A. and Bensouilah, M. (2023). The Sea Urchin *Sphaerechinus granularis* (Lamarck, 1816) from the Mediterranean Sea: A New Natural Source of Antibacterial and Antioxidant Molecules. Indian Journal of Animal Research. 57(8): 1051-1058. doi: 10.18805/IJAR.BF-1485.

Submitted: 27-12-2021 **Accepted:** 05-04-2022 **Online:** 25-04-2022

MATERIALS AND METHODS

Biological materials

The study was carried out in April 2019, 200 specimens *Sphaerechinus granularis* sea urchins (diameter between 30 and 50 mm) were harvested from coastal areas of El-Kala (Cap Rosa) in the south-eastern Mediterranean. The sea urchins collected were transported alive to the laboratory in a cooler with oxygenated seawater. In the laboratory, the test was opened and the female gonads were removed. The bacterial strains used were reference strains of Gram negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATTC 700603, *Pseudomonas aeruginosa* ATCC 27853) and Gram positive bacterial strains (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212). These strains were provided by Dr. Lamia Benhalima and Dr. Saber Belhaoues (laboratory of ecobiology of marine environment and coastlines (EMMAL), Badji Mokhtar University, Algeria). All manipulations and products are produced and provided by the EMMAL Laboratory under the direction of Professor Bensouilah Mourad. Research period is 7 months.

Crude extract preparation

The aqueous extract preparation was carried out according to Bragadeeswaran *et al.* (2013), the gonads was macerated in distilled water for 12 hours. The methanolic extract preparation was carried out according to Sidiqi *et al.* (2019), the gonads were macerated in methanol for 24 hours (3x). The two were extracts are filtered, evaporated, lyophilized and stored at - 20°C for further analysis.

Screening of consultants

The gonads was screened for the presence of different classes of secondary metabolites including alkaloïds, anthocyanins, anthraquinones, flavonoids, leuco-anthocyanins, mucilages, phenols, reducing compound, saponins, steroids, tanins and triterpenoids, using the colorimetric methods described by Edeogal *et al.* (2005) and Karumi *et al.* (2004). The presence of secondary metabolities was determined by precipitation, turbidity, or color change reactions.

Determination of gonadal compounds

Estimation of total carotenoids

The total carotenoids was determined using the method of Susan and Damodaran (1997) by the use of acetone for extraction. Absorbance was measured at 455 nm. The results were expressed as µg/g of gonads.

Estimation of vitamin C and E content

Vitamin C was determined using the method of Jagota and Dani (1982) by the use of Folin-Ciocalteu reagent. Absorbance was measured at 760 nm and ascorbic acid was used as standard. The results were expressed as µg/g of gonads. The vitamin E was determined using the method of Martinek (1964) by the use of 2,4,6-tripyridyl-s-triazine (TPTZ) reagent. Absorbance was measured at 600 nm and α-tocopherol was used as standard. The results were expressed as mg/g of gonads.

Characterization of extracts

DPPH radical scavenging assay

The antioxidant potential was determined using the method of Yen and Chen (1995) by the use of 2, 2-diphényl 1-picrylhydrazyle reagent (DPPH) reagent. The absorbance was measured at 515 nm and the butylated hydroxytoluene (BHT) and ascorbic acid was used as a reference standard. The antioxidant activity of the extract was expressed as IC_{50}.

Ferric reducing/antioxidant power assay (FRAP)

The antioxidant potential was determined using the method of Deighton *et al.* (2000) by the use of 2, 4, 6-tripyridyl-s-triazine (TPTZ) reagent. The absorbance was measured at 593 nm and ascorbic acid was used as a reference standard, the results were expressed as µM.

Determination of total phenolic and flavonoids content

The total phenolic content was determined by using the method of Singleton and Rossi (1965) by the use of Folin-Ciocalteu reagent. Absorbance was measured at 765 nm and gallic acid was used as standard. The results were expressed as mg of gallic acid equivalents/g of extract. Flavonoids content was determined according to the procedures described by Arvouet-Grand *et al.* (1994) by the use of aluminum trichloride ($AlCl_3$) reagent. The absorbance was measured at 430 nm. Quercetin was used as standard and results were expressed as mg of quercetin equivalents/g of extract.

Antibacterial activity

The antibacterial activity was evaluated by the method of diffusion, as described by Celiktas *et al.* (2007). The extracts was dissolved in 2% dimethyl sulfoxide (DMSO) at 200 mg/ml, a young bacterial suspension was adjusted to 0.5 McFarland and then diluted and spread on Petri dishes containing Mueller-Hinton agar. The extract was deposited at 50, 100 and 200 mg in the wells. The Petri dishes were incubated in oven, at 37°C for 24 hours. The inhibition evaluation is carried out by measuring the diameter of the inhibition zone around each well. DMSO was used as negative control and antibiotic discs of gentamicin (10 µg) as a positive control.

Determination of the minimum inhibitory and bactericidal concentration

The determination of the minimum inhibitory concentration (MIC) and of the minimum bactericidal concentration (MBC) was carried out according to the method described by Benhalima *et al.* (2019, 2020). The extracts was dissolved in DMSO (2%) at 1000 mg/ml and then diluted as per the requirement. The range of concentrations chosen was from 0 to 500 mg/ml. After adjusted to 0.5 McFarland and dilution

of the inoculums, 1 ml of the diluted inoculums with broth Mueller-Hinton were added to 1 ml of each extract concentration, the tubes were incubated in an oven at 37°C for 24 hours. The MIC was defined as the lowest dilution with negative growth. To determine the MBC, a 10 µL from those tubes, which did not show any visible growth in MIC assay, was cultured on nutrient agar and incubated at 37°C for 18 to 24 hours. The lowest concentration of extract producing no growth was considered to be the MBC. Non-inoculated broth Mueller-Hinton was used as the negative control and broth Mueller-Hinton without the addition of extract as the positive control.

Statistical analysis

The data is expressed in mean values±standard deviation of the mean (SD), all measurements were done in triplicate. Statistical analysis of the data was performed using XL STAT 2014 software and the normal distribution was verified by applying the Shapiro-Wilk test, making it possible to choose non-parametric methods for the statistical analysis. Analysis of variance (Kruskal-Wallis test) was used to compare between species and concentrations. Mann-Whitney test was used to compare antioxidant activity with two standards, as well as the concentration of flavonoids and polyphenols in the two extracts. The tests were performed at a significance level of 0.05.

RESULTS AND DISCUSSION

Yields in dry extracts

The results indicated that, the aqueous extract produced the highest yield (6.20%) compared to the methanolic extract (5.10%). Similarly, the color was light orange for the methanolic extract and dark orange for the aqueous extract. This difference can be explained by the amount of total extractable compounds which is inversely proportional to decreasing polarity of the solvent used (Belhaoues *et al*., 2020).

Screening

Screening is presented in Table 1, the results revealed the presence of saponosides, mucilages, alkaloids, combined anthraquinones (C-heterosides), polyphenols and flavonoids. However, steroids, triterpenoids, tannins, anthocyanins, combined anthraquinones (O-heterosides), free anthraquinones, leucoanthocyanins and reducing compounds, were not present. Likewise, several scientific studies have shown that sea urchin gonads offer numerous types of components with high medical value. The gonads are rich in bioactive components, such as polyunsaturated fatty acids (Robinson and Blair, 2008), carotenoids (Matsuno and Tsushima, 2001), phospholipids (Shikov *et al.,* 2012), sulfated fucans (Biermann and Mourão, 2002) and active polysaccharides (Shikov *et al.,* 2018). The results from this study were more or less comparable to those of Akerina *et al.* (2015) which indicated the presence of steroids and triterpenoids. On the other hand, Sidiqi *et al.* (2019) reported the absence of alkaloids and phenols on sea urchin *Diedema setosum*. These differences in composition may be due to the detection capacity of the chemical test as some tests are unable to detect low amounts (Artini *et al.,* 2013). Further, it could be due to the different environmental conditions and also due to the different maturity stages of sea urchin gonad, which was used as research material (Darsono, 1986) as well as the genera and species.

Estimation of carotenoids

The composition of carotenoids is represented in Table 2, the carotenoids contents were 15.84±0.01 µg/g of gonads. Carotenoids are widely distributed, naturally occurring pigments, usually red, orange, or yellow in color (Matsuno and Hirao, 1989). The carotenoids concentrations are high in the reproductive organs, which suggests their importance in reproduction (Goodwin, 1984). In our results, the concentrations obtained are more or less variable compared to those reported by Griffiths and Perrot (1976); Tsushima and Matsuno (1990); Lamare and Hoffman (2004), they recorded concentrations between 5 and 1870 µg/g of gonads. This difference could be attributed to the methodology adopted, species used, feeding, environmental conditions as well as gametogenesis. Borisovets *et al.* (2002) reported that the pigments of the gonads, were high at spawning or during active gametogenesis. In general,

Table 1: Screening of the gonads of the sea urchin *Sphaerechinus granularis*.

Screening		Results
Alkaloïds		+
Phenols		+
Flavonoids		+
Mucilages		+
Saponins		+
Combined	C-hétérosides	+
anthraquinones	O-hétérosides	-
Free anthraquinones		-
Tanins	Catéchique	-
	Gallique	-
Anthocyanins		-
Leuco-anthocyanins		-
Reducing compound		-
Steroids		-
Triterpenoids		-

(-): Not detected - (+): Detected.

Table 2: Characterization of the gonads of the sea urchin *Sphaerechinus granularis*.

Metabolite	Concentration
Carotenoids (µg/g)	15.83±0.01
Vitamin C (µg/g)	61.77±0.71
Vitamin E (mg/g)	112.74±0.10

Each value is presented as mean±SD (n=3).

animals do not synthesize carotenoids and those found in bodies of animals are an accumulation of carotenoids from the food (Griffiths and Perrott, 1976). The work of McLaughlin and Kelly (2001) indicated that the diet rich in microalgae improves gonad quality.

Estimation of vitamin C and E content

The composition of vitamin C and E is represented in Table 2, a high vitamin E (112.74±0.10 mg/g) was found, compared to vitamin C (61.77±0.71 µg/g). The gonads are a source of vitamins, minerals and other micronutrients, the diet of the sea urchin can influence biochemical composition of the gonads (Chen *et al.,* 2010). Sea urchin roes are rich in vitamins (Jinadasa *et al.,* 2016) as the food source comes from different types of algae (Akerina *et al.,* 2015). Vitamin E is an antioxidant, which serves to neutralize free radicals and prevent lipid oxidation (Vasanthi *et al.,* 2012). The abundance of vitamin E in sea urchins has already been highlighted in the work of Salma *et al.* (2016) of the species *Diadema setosum* (23.47 mg/100 g of gonads). Also, the work of De-Quirós *et al.* (2001) reported the presence of vitamin C in *Paracentrotus lividus* (26.57±1.50 mg/100 g of gonads).

Antioxidant capacity

The results obtained with DPPH and FRAP methods are presented in Table 3, the antioxidant capacity is of the order of IC_{50}= 02.690±0.37 µg/µg of DPPH by methanolic extract and IC_{50}= 05.87±0.27 µg/µg of DPPH for the aqueous extract. Methanolic extract is the most active with the lowest EC_{50}, because a low IC_{50} value represents a high antioxidant activity. The reducing power of the two extracts showed better activity of the methanolic extract compared to the aqueous extract, the methanolic extract showed higher FRAP than ascorbic acid (800±0.98 µM). Data presented no significant difference ($p>0.05$) between extracts and standards, which indicates a appreciable antioxidant activity. The strong antioxidant activity of the methanolic extract would be due to solvent, which is able to destroy cell wall and causes the components in the cell to disintegrate and dissolve in solvents (Lapornik *et al.,* 2005). Various results indicated that sea urchins generate has many components that act as antioxidants (Jazayeri, 2012), the gonads are rich in antioxidants like polyhydoxylated naphthoquinone and echinochrome A (Aminur Rahman *et al.,* 2014). The antioxidant potential of gonads has already been reported in *Strongylocentrotus droebacheinsis* (Mamelona and Peltetier, 2010), *Strongylocentrotus nudus* (Shang *et al.,* 2018), *Tripneustes gratilla* (Chen and Hwang, 2014) and *Stomopneustes variolaris* (Archana and Babu, 2016). This good antioxidant capacity is the fact that the gonads contain carotenoids and polyphenols which possess potent antioxidant activity (Archana and Babu, 2016). The consumption of gonads of sea urchin is associated with anti-inflammatory, anti-atherosclerotic and anti-carcinogenic activities (Mamelona and Peltetier, 2010). Likewise, Extracts from the gonad of sea urchin may act as photoprotectants, mitigating the damaging effects of UV radiation and increasing larval survival rates (Lamare and Hoffman, 2004).

Total phenolic and flavonoids content

The results obtained are represented in Table 3, the total polyphenol contents of extracts were 2.22±0.21 and 3.43± 0.09 mg/g for aqueous and methanolic extracts, respectively. The flavonoid contents of extracts were 0.95±0.07 mg/g for aqueous extract, while the corresponding content for the methanolic extract was 1.70±0.04 mg/g. In our results, there were higher amounts of polyphenols and flavonoids in methanol extract than in aqueous extracts. The data presented no significant difference ($p>0.05$) between extracts. However, this finding does not resolve the difference both in terms of quantity and quality of bioactive molecules. Methanol showed a little bit better characteristic as a solvent for the extraction polyphenols and flavonoids than water. The polyphenols are a class of low molecular weight secondary metabolites. The polyphenolic compounds are also found in sea urchin gonads, they are considered important as bioactive dietary compounds with putative health benefits, as they are able to terminate free radicals and chelate metal ions, which are capable of catalyzing formation of ROS (Mamelona and Peltetier, 2010).

Antibacterial activity

The results obtained are represented in Table 4, the DMSO at 2% is adequate and does not exhibit any impact on the normal process of growth of the reference strains. The bacterial resistance could be very critical, the gentamicin (10 µg) tested as a positive control has indicated inhibition zones between 17.5±01.37 and 25.66±01.21 mm. The methanolic extract showed considerable antibacterial activity at 200 mg against *Klebsiella pneumoniae* (28.33±0.52 mm), *Escherichia coli* (21.46±0.75 mm), *Staphylococcus aureus* (19.6±0.59 mm) and *Enterococcus faecalis* (18.33±0.57 mm).

Table 3: Characterization of the aqueous and methanolic extracts from gonad of the sea urchin *Sphaerechinus granularis.*

Dosage	Extracts		Standards	
	Aqueous	Methanolic	Ascorbic acid	BHT
DPPH IC_{50} (µg/µg of DPPH)	05.87±0.27	02.69±0.37	02.60±0.32	02.70±0.69
FRAP (µM)	363.33±45.09	12600±164.97	803.88±15.05	-
Polyphenols (mg/g of gallic acid equivalent)	02.22±0.21	03.43±0.09		
Flavonoids (mg/g of quercitin equivalent)	0.95±0.07	01.70±0.04		

Each value is presented as mean±SD (n=3).

Absence of superscript indicates no significant differences in the same row ($p<0.05$).

No zone of inhibition was observed for the species *Pseudomonas aeruginosa*. The data presented significant difference ($p<0.05$) between the different bacterial strains and concentrations. According to Tiwari *et al.* (2014), the gram-negative bacteria showed less sensitivity and this may be due to their extra-lipopolysaccharide and protein cell wall that provides a permeability barrier to the antibacterial activity. However, this result was not observed in our study since the extract had an almost similar effect on the gram negative than gram positive. These results suggests that the presence of bioactive molecules have a broad-spectrum antibacterial activity (Belhaoues *et al*., 2017). The results of our study differs research reported by Bragadeeswaran *et al.* (2013); Lisa Ah Shee Tee *et al.* (2017); Sidiqi *et al.* (2019) and El-Sayed *et al.* (2020) where more or less significant zones of inhibition were found. This difference could be caused by several reasons including, the extract concentration (Ariyanti *et al.,* 2012), the size and gender of gonads. As well, the genus and species of sea urchin and maturity. The gonads of *Sphaerechinus granularis* used in this study is in a mature phase, however some sea urchins were in the pre-mature phase. According to Darsono (1986), the gonadal maturity can not only be determined by size. Likewise, the solvent used for the extraction plays an important role. The methanol was able to extract components derived from alkaloids, phenols and carotenoids. The saponin compounds have potential as antibacterial because they are polyphenol compounds that can inhibit bacteria by damaging the permeability of bacteria cell membranes (Sikkema *et al.,* 1995). The biological function of saponins which are related to the system of self-defense against marine fungi, predators and parasites (Pranoto *et al*., 2012). Likewise, the flavonoids are one of the polar phenol compoundshave high antibacterial activity (Darsana *et al*., 2012). It should be noted, however, that the attribution of the antibacterial activity of a complex mixture to a single compound is subjective (Belhaoues *et al*., 2020). Because the possible synergistic effect of the phenolics compounds with one another (Lopes-Lutz *et al*., 2008). Also, no antibacterial effect was found for the species *Pseudomonas aeruginosa*, it is unlikely that this is due to the low concentration of bioactive molecules and ability to develop resistance against multiple classes of antimicrobials which is alarming and concern (Sekhri *et al.,* 2021). The aqueous extract did not indicate antibacterial activity.

Minimum inhibitory and bactericidal concentration

The results obtained are represented in Table 5, for the methanolic extract, the lowest MIC was observed for the species *Klebsiella pneumonia* (50 mg/ml). Values between 60 and 90 mg have been observed for *Escherichia coli*, *Staphylococcus aureus* and *Enterococus faecalis* species. For MBC, it is around 400 mg/ml for *Staphylococcus aureus* and *Enterococus faecalis* and 100 mg/ml for *Escherichia coli* and *Klebsiella pneumonia*. Concerning the aqueous extract, the MIC and MBC could not be obtained, due to the

Table 4: Diameters of the zones of inhibition of the reference strains with respect to the aqueous and methanolic extracts.

Reference strains	Inhibition zone of methanolic extract (mm)			Inhibition zone of gentamicine 10 µg (mm)
	50 mg	100 mg	200 mg	
Escherichia coli ATCC 25922	$10.53\pm0.50^{a,*}$	$16.5\pm0.5^{a,\$}$	$21.46\pm0.75^{a,\#}$	25.33±01.52
Klebsiella pneumoniae ATCC 700603	$11\pm01^{b,*}$	$16\pm01^{b,\$}$	$28.33\pm01.52^{b,\#}$	25.33±0.57
Pseudomonas aeruginosa ATCC 27853	00±00*	00±00$	00±00#	19.66±0.57
Staphylococcus aureus ATCC 25923	$08.5\pm0.5^{c,*}$	$13.00\pm01^{c,\$}$	$19.6\pm0.59^{c,\#}$	18.33±01.52
Enterococcus faecalis ATCC 29212	$08.33\pm0.57^{d,*}$	$11.33\pm0.57^{d,\$}$	$18.33\pm0.57^{d,\#}$	17.66±01.15

Aqueous extract: No effect detected. 4

Each value is presented as mean±SD (n=3).

DMSO at 20%: No effect detected. Presence of the same exponent (letter) indicates significant differences in the same row.

Presence of the same superscript (sign) indicates significant differences in the same column.

Table 5: Minimum inhibitory and bactericidal concentration of the aqueous and methanolic extracts.

Reference strains	Concentration (mg/ml)				Standard according to Marmonier (1990)	
	MIC	MBC	MBC /MIC	Antibacterial activity	Bactericidal effect	Bacteriostatic effect
Escherichia coli ATCC 25922	60	100	1.66	Bactericidal	MBC/MIC ≤ 4	MBC/MIC> 4
Klebsiella pneumoniae ATCC 700603	50	100	2	Bactericidal		
Pseudomonas aeruginosa ATCC 27853	> 500	> 500	-	-		
Staphylococcus aureus ATCC 25923	80	400	5	Bacteriostatic		
Enterococcus faecalis ATCC 29212	90	400	4.44	Bacteriostatic		

Aqueous extract: No effect detected.

Negative control: No visible growth.

Positive control: Visible growth.

turbidity of the extract and the range of concentrations chosen which could be lower than the MIC. For the *Pseudomonas aeruginosa* species, the MIC is over 500 mg/ml. This species is a danger to public health because of its rapid growth, plasticity of its genome and is naturally resistant to several antibiotic families (Benhalima, 2016). The comparison of the MBC/MIC ratio with the intrinsic values of the bioactive molecules proposed by Marmonier (1990), allowed us to indicate that the methanolic extract has a bactericidal effect against *Escherichia coli* and *Klebsiella pneumonia* and bacteriostatic against *Staphylococcus aureus* and *Enterococcus faecalis*. Unfortunately, it was impossible to compare the results obtained, because very little work on the MBC/MIC ratio of sea urchins has been carried out.

CONCLUSION

The present study showed, that the gonads of sea urchin *Sphaerechinus granularis* contain the saponosides, mucilages, alkaloids, combined anthraquinones (C-heterosides), polyphenols and flavonoids. The dosage of vitamin C and E, carotenoids, polyphenols and flavonoids indicated that the gonads are more or less rich in secondary metabolities. Moreover, the antioxidant activity is promising. The antibacterial test indicates that the methanolic extract exhibited an antibacterial activity especially against *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus* and *Enterococcus faecalis.* Also, the methanolic extract has a bactericidal effect against *Escherichia coli* and *Klebsiella pneumonia* and bacteriostatic against *Staphylococcus aureus* and *Enterococcus faecalis*. This result suggested that gonads of sea urchin might be beneficial as a functional food. Consequently, further works are necessary to identify the main antibacterial agents and the mechanisms of action of the single component. Further research, including purification and isolation of the bioactive molecules such as polyphenol, saponoside, carotenoids, *etc.*, would be worthwhile in order to establish their real potential on pathogenic and resistant nosocomial strain.

Conflict of interest: None.

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