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AN EXTRACT OF LION FISH (*Pterios miles*) BONE IS EFFECTIVE AT KILLING (*Pseudomanas*) AND (*Klebsella penumania*) GERM COMPARED TO SOME INDUSTRIAL ANTIBIOTICS

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This research aims to study the effectiveness of an extract from toxic lionfish spines (*Pterios miles*) against two types of germs (*Pseudomanas*) and (*Klebsella penumania*) by analyzing the toxic substance for inhibition of growth of the bacteria on solid media. The spines were sampled and the toxic compound from the spines was extracted for analysis.

The experiment was conduct with one replicate of each concentration for periods approved in the research, and the results of the improvement of the germs strains were recorded against the concentration impregnated tablets that have been approved for the toxic extract, also against the industrial antibiotics.

Where the diameter of the inhibition circles was measured in centimeters around each tablet, and a comparison was made between each of the effectiveness of the toxic extract and the effectiveness of industrial antibiotics in order to evaluate and determine which is more effective.

Found out through this research the inhibitory efficacy of lionfishs spines extract against the growth germs types in this study, but it differed according to the concentrations used and achieved the best result in the effectiveness of the extract at the cured concentration. In addition, the toxic extraction was better than the used antibiotics.

Keywords: Scorpaenidae; *Pterios miles*; *Pseudomanas*; *Klebsella pneumonia*.

1. INTRODUCTION

Seas and oceans are considered to be the largest natural ecosystem, and very important because it is a huge store for many resources, in addition to the fact that it is used for food for an ever increasing population [1]. In addition, it is characterized by a huge biodiversity of animals and plants, which are estimated to be twice as diverse as those on land.

This diversity gives the marine creature a big chance to discover the bioactive substance.

Oceans are rich sources of new chemical compounds produced by marine life, have not ever completely

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discovered [2]. Natural products with biochemical activity are also an important source in the pharmaceutical industry.

The Scorpaenidae is a marine fish that are home to many of the world's most venomous fish species. It include a hundreds of individuals. They are widespread in tropical and temperate seas, but are mostly found in the Indian and Pacific oceans.

The genus Pterios is a venomous marine fish, known as the lionfish, native to the Indian and Pacific oceans. These fish differ in color from a color that resembles a red color to a brown-brown or gray color. They have many thin, dark-colored spines on their heads and bodies, and poisonous barbed fin rays [3].

The common lionfish (Pterois miles), or the dragon fish, is also known as the common sea roosterfish. It is native to the western Pacific-Indian regions.

Common lionfish grow to a length of about 35 cm. The dorsal fin contains 13 long, strong spines and between (11-9) soft rays. The anal fin has three long spines and six or seven soft rays. The dorsal fin is feathery and the pectoral fins are wing-like with broad, discrete rays.

Lionfish are known for their venomous fin rays, the strength of their venom makes them an excellent and dangerous predator for anglers and divers [3].

Lionfish venom contains acetylcholine, a neurotoxin that affects neuromuscular transmission [4]. Lionfish venom can cause systemic effects such as severe pain, nausea, vomiting, fever, difficulty breathing, convulsions, dizziness, redness in the affected area, headache, numbness, heartburn, diarrhea, and sweating. In rare cases, these stings can cause temporary paralysis of the limbs, heart failure, and even death [5].

2. THE IMPORTANCE AND OBJECTIVES OF THE RESEARCH

The importance of the research is evident in the following points:

- ☒ The study of the effectiveness of the extract of lionfish spines against germs that are pathogenic to humans is consider one of the important researches in the field of marine biotechnology that is directed towards obtaining natural materials of therapeutic importance from marine organisms and using them as non-traditional antibiotics to treat human diseases.
- ☒ The research deals - for the first time - with studying the effectiveness of the extract of the toxic substance of lionfish spines on some types of pathogenic microorganisms and the possibility of using it in the pharmaceutical field later.
- ☒ The research contributes to studying the possibility of benefiting from these non-economic fish (due to their deadly toxicity) by marketing them to the competent authorities in the pharmaceutical field.

Through this, the research objectives are:

- ✓ Determination of the qualitative composition of the lionfish in the marine waters of the coast of Latakia and the morphological - standard characteristics.
- ✓ Testing the efficacy of different concentrations of the (organic, toxic) extract of lionfish spines against two types of germs pathogenic to humans (*Pseudomanas* and Klebsella pneumonia).
- ✓ Determine the most effective concentrations as antibiotics, and compare them with each of the industrial antibiotics (Dorpenem, Tetracycline, Cefoperazone) currently used

Through this, the research objectives are:

3. RESEARCH MATERIALS AND METHODS

3.1 Research Area

The research was conducted in the northwest of the Syrian Arab Republic, in the port of Al-Sayd and Al-Nuzha on the shore of Latakia city. Where this area represents a natural environment for the presence and distribution of the species *Pterios miles* of the family Scorpaenidae.

3.2 Sample Collection

Fish samples: (*Pterios miles*) were collected during the year 2021-2022 at a rate of once per season (summer / August 2021, autumn / November 2021, winter / January 2022, spring / April 2022 /) from different locations in the research area. Where samples of different sizes of the aforementioned fish species were collected by local fishing methods (nets and cages) at depths ranging between 25-50 meters. The lab work was conducted at Tishreen University for the morphological study, to obtain extracts of poisonous thorns and to study the biological activity of the extract against isolated pathogens.

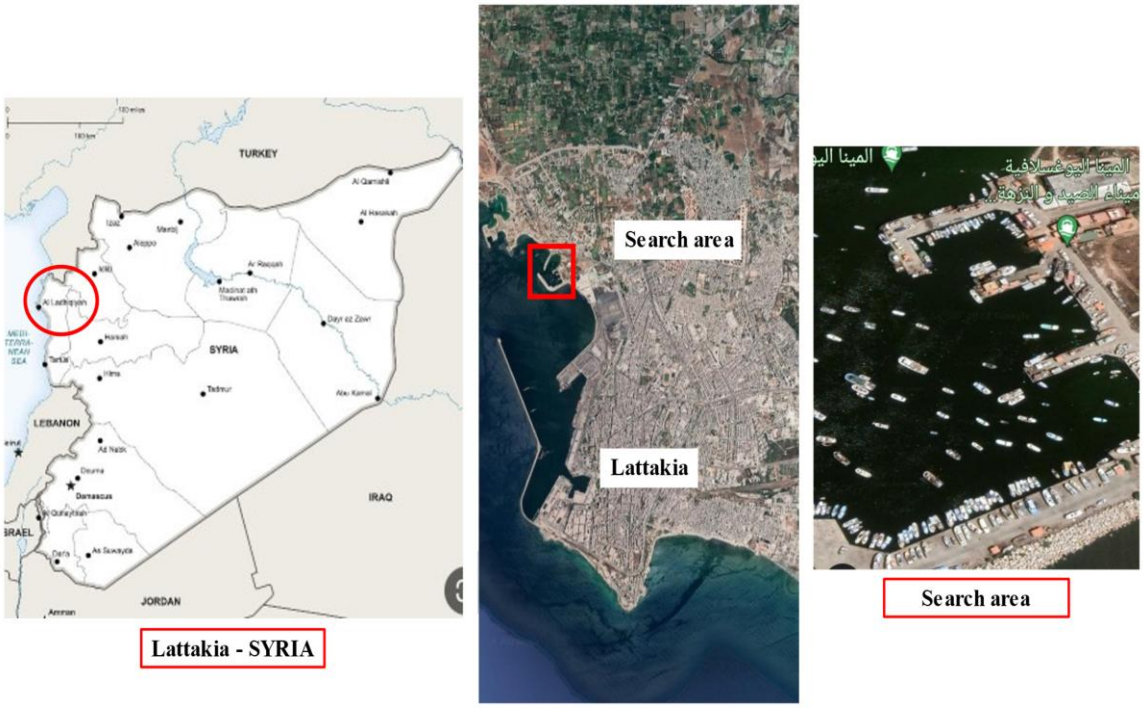

Fig. 1. The research area within Latakia Governorate, Syria

3.3 Laboratory Work

The morphological characteristics studied and morphometric measurements taken: (weight, total length, standard length, maximum height) and the genus was determined.

After that, the collected spins were weighed with a sensitive electronic balance, and ground with a sterile blender in order to increase the contact surface with the used organic solvent (methanol) according to the following method:

Samples were taken from the spines of lionfish, to extract the toxic compound from them with methanol; including /1% of acetic acid/ for 5 minutes in a mixer, then the samples were centrifuged with a mixture of 3000 revolutions/ for 15 minutes.

The extraction was re-extracted and the extract collected, concentrated to a certain volume by rotary evaporator, and re-dissolved in 1% aqueous acetic acid.

The lipide were removed from the aqueous extracts with an equal volume of chloroform, then the chloroform removed with a separation funnel, the aqueous organic compound filtered, and a rotary evaporator (UNEP, 2003) concentrated the extracted sample.

Then different concentrations (10 - 20 - 30 - 40 - 50) mg/ml were prepared from the crude extract.

Pathogenic bacterial samples were isolated from different human pathological samples taken from the germ Laboratory at Tishreen University Hospital, and the following culture media (Nutrient Agar, Eosin methylene blue, Mac Conky, Muller Hinton) were used to confirm the identity of the studied pathogenic bacterial isolates using culture characteristics, staining, microscopy, and chemical tests.

The isolated pathogenic germ were cultured on general and selective media, and stained by Gram stain. Then, they were subjected to differential chemical tests to determine the germ strains of each of them. Then they were grown using the aforementioned culture media for 24 hours at a temperature of 37 degrees Celsius, after that a smear was taken with a sterile knotted needle and the needle was immersed in sterile tubes containing physiological serum and the tubes were shaken to ensure homogeneity of the bacterial suspension, then the tubes were poured by pouring method onto plates containing medium Muller Hinton, and the dishes were stirred with a cyclonic motion to ensure uniform brushing on the surface of the medium, and left for 5 minutes, then the excess was removed from the suspension, and the dishes were set aside for 10 minutes to dry.

Sterile filter discs with a diameter of 6 mm were used, which were dipped in the extracts from the spines at concentrations: (raw - 10 - 20 - 30 - 40 - 50) mg/ml. Discs impregnated with sterile water only were used as a negative control, and discs of some antibiotics with a diameter of 6 mm belonging to groups were used. Different as a positive control were chosen according to the bacterial strain and the concentrations currently in circulation, Table 1.

Table 1. Some of the antibiotics used in the research

Gode	Antibiotics
DOR	Dorpenem
TET	Tetracycline
CPZ	Cefoperazone

After that, these tablets were placed with sterile and flaming forceps in dishes that were prepared according to the type of bacterial strain and left for an hour at laboratory temperature to allow the active substance to spread within the medium, and then transferred to an incubator at 37 °C for 24 hours, where the inhibition rings appeared. Muller Hinton media around the discs is clear evidence of inhibition of bacterial growth, and the diameters of inhibition recorded with a millimeter ruler.

The experiment conducted with one repetition of each concentration for the periods approved in the research, and the results of the sensitivity of the bacterial strains towards the approved concentrations of the toxic extract, as well as towards the antibiotics mentioned above, recorded.

The diameter of the inhibition circles (cm) measured around each tablet, and a comparison made between each of the efficacy of the toxic extract and the efficacy of the antibiotics in order to evaluate and determine which is the most effective.

4. FINDINGS AND DISCUSSION

4.1 Morphological Characteristics of the Lionfish (Bennett, 1828) Pterios Mils

It characterized by the presence of alternating red and black stripes. The dorsal spines and the pectoral and ventral fins alternately streaked with red, black and pink colors. The dorsal, anal and caudal soft rays have a series of dark spots, Fig. 2.

The body is slightly compressed, the vertical angles bear spiny projections, the mouth is large with villi-shaped teeth, the dorsal fin spines are longer than the body, the anal and caudal fins are rounded, while the pectoral fins are wing-like and have separate broad rays, and this corresponds to: [6-11].

Tables 2, 3, 4, 5 show the morphometric measurements of the lionfish Pterios mils for forty samples taken during four distinct periods from the year 2021-2022.

From Tables 2, 3, 4, 5, the following appears:

The weight of the studied individuals of lionfish (Pterios mils) ranged during the research period between (27.3-292.6) g, and their total lengths ranged between (15.3-27.4) cm, and their standard lengths ranged between (11.2-20.4) cm, and their maximum height values ranged between (3.1 - 8.8) cm.

Fig. 2. The general shape of Pterios mils caught in the marine waters of Latakia Governorate, Syria during the year 2021-2022

Table 2. Morphological measurements of the lionfish (Pterios mils) for samples taken in the summer season (August) of the year 2021

Genus	Height (cm)	Standard length (cm)	Total length (cm)	Wight (g)	Sample number
M	6.1	18.5	24.9	207.1	I
M	8.8	20.4	27.1	292.6	2
M	4.5	14.9	19.6	80.2	3
F	3.9	12.6	16.9	38.2	4
M	4.1	11.2	15.3	27.3	5
F	5.4	14.3	20.1	80.3	6
M	4.5	13.7	18.5	55.4	7
F	4.8	13.2	18.1	51.7	8
M	5.1	12.9	17.4	53.2	9
M	4.7	12.9	17.2	54.5	10

Table 3. Morphological measurements of lionfish (Pterios mils) for samples taken in the fall season (November) of the year 2021

Genus	Height (cm)	Standard length (cm)	Total length (cm)	Wight (g)	Sample number
M	8.1	18.2	24.7	248.5	1
F	4.1	13.3	17.4	47.1	2
M	5.4	15.4	20.9	95.2	3
F	4.9	14.0	18.7	66.6	4
M	4.8	14.3	18.9	67.2	5
M	4.9	13.8	18.9	52.8	6
F	4.7	12.8	17.9	52.5	7
M	5.1	14.1	18.8	67.1	8
M	4.8	13.6	18.4	59.6	9
F	5.4	16.3	21.2	110.4	10

Table 4. Morphological measurements of lionfish (Pterios mils) for samples taken in the winter season (January) for the year 2022

Genus	Height (cm)	Standard length (cm)	Total length (cm)	Wight (g)	Sample number
M	3.2	11.4	15.6	35.3	1
F	4.9	14.0	18.9	70.9	2
F	4.6	13.2	18.1	65.7	3
M	7.4	20.3	27.4	259.8	4
F	5.8	15.4	21.2	122.3	5
F	5.3	15.3	20.5	129.0	6
F	5.4	15.4	21.2	137.4	7
M	5.2	15.2	20.3	116.4	8
M	4.7	13.8	18.8	87.1	9
F	4.6	12.9	17.5	73.1	10

Table 5. Morphological measurements of lionfish (Pterios mils) for samples taken in the spring season (April) for the year 2022

Genus	Height (cm)	Standard length (cm)	Total length (cm)	Wight (g)	Sample number
F	4.8	16.1	21.4	90.0	1
F	6.3	16.4	21.5	105.0	2
F	5.1	13.9	18.5	60.0	3
F	6.6	16.4	21.4	110.0	4
M	5.2	15.6	20.6	85.0	5
F	5.3	16.9	20.5	110.4	6
M	4.9	15.1	19.2	53.1	7
M	5.6	16.1	21.3	95.9	8
F	6.8	16.1	20.7	69.6	9
M	3.1	11.3	15.4	45.3	10

4.2 Efficacy of *Pterios miles* Extract Against the Studied Pathogenic Bacteria

The effectiveness of multiple concentrations of this species' thorns extract against the bacteria approved in the research studied, and the results showed that the extract showed clear effectiveness against the studied bacteria in different proportions depending on the concentration of the extract.

We will review the effectiveness of the extract against each type of bacteria taken separately:

☒Pseudomonas:

These germs are commonly found in the environment, and they cause infections in the blood, lungs, and other parts of the body after surgical operations. They are also responsible for blood poisoning of burn patients, and characterized by their great resistance to antibiotics.

Microscopic examination of these bacteria showed straight, slightly curved, movable bacilli, which were Gram-negative. They also appeared by culture on nutrient agar medium in the form of gray-colored colonies with irregular edges, characterized by being mucous and emitting an odor similar to that of bitter almonds.

These bacteria characterized by the following biochemical characteristics: they do not ferment lactose - methyl red negative - indole negative.

Table (6) shows the diameters of the rings of bacterial growth inhibition resulting from thistle extract in four different periods of the year 2021-2022.

A chart was draw showing the average diameters of the bacterial growth inhibition rings resulting from the toxic extract of Pterios mils against *Pseudomanas* bacteria, Fig. (3).

We noticed from Fig. (3) that the values of the inhibition diameters of the thorns extract ranged between (2.2) cm at the crude concentration and (0.9) cm at the (50) concentration.

Table 6. Diameters of bacterial growth inhibition rings, estimated in cm, resulting from Pterios mils extract of thistles against *Pseudomanas*

Inhibition diameter (cm)				Concentrations (mg/ml)
Spring 2022	Winter 2022	Fall 2021	Summer 2021	
2.7	2.0	2.1	2.0	raw
1.7	1.5	1.7	1.8	10
1.0	1.4	1.5	1.6	20
0.9	1.4	1.4	1.6	30
0.7	1.0	1.2	1.4	40
0.7	0.8	1.0	1.2	50

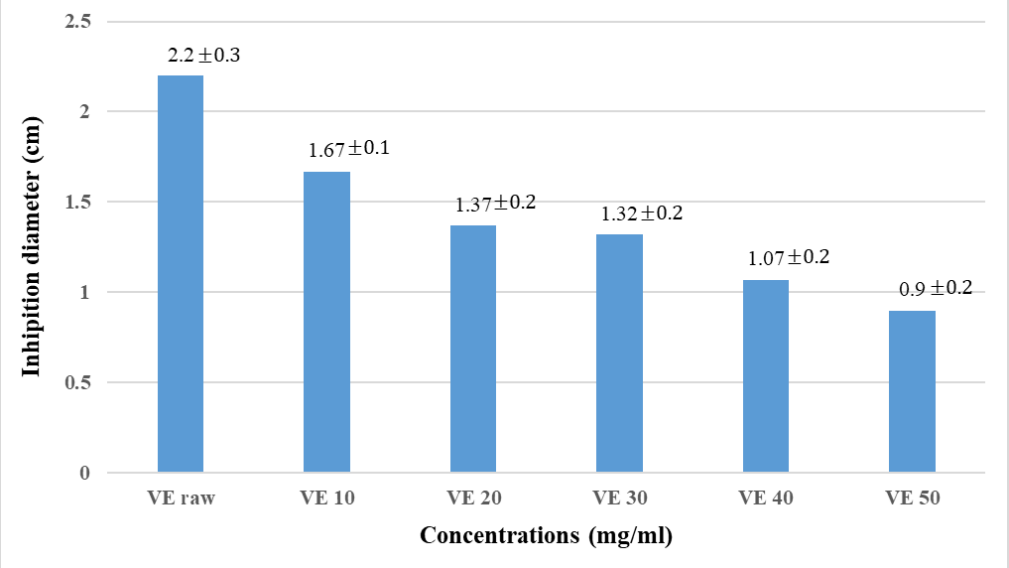

Fig. 3. The average diameters of bacterial growth inhibition rings, estimated in cm, resulting from the toxic extract Lionfish's Pterios mils towards *Pseudomanas*

☒Klebsella pneumonia:

It considers one of the opportunistic bacteria that appears in immunosuppressed patients, and it is resistant to antibiotics. It causes pneumonia, urinary tract infection, and wound infection.

Microscopic examination of these bacteria showed short, non-motile bacilli surrounded by a capsule, which are Gram-negative, with round mucous colonies of pink color when cultured on EMB medium.

These bacteria characterized by the following biochemical characteristics: fermentation of lactose, glucose, and sucrose - indole-negative - oxidase-negative.

Table (7) shows the diameters of the bacterial growth inhibition rings resulting from thistle extract in four different periods of the year 2021.

A chart was drawn showing the average diameters of the bacterial growth inhibition rings resulting from the toxic extract of Pterios mils against Klebsella pneumonia, Fig. (4).

We noticed from Fig. (4) that the values of the inhibition diameters of the thorns extract ranged between (2.92) cm at the crude concentration and (1.32) cm at the (50) concentration.

4.3 Comparison of the Effectiveness of the Toxic Extract with the Effectiveness of Some Antibiotics Against the Studied Pathogenic Germs

Three antibiotics Table (1), belonging to different groups, were used as a positive control. They chose according to the bacterial strain, and their effectiveness studied at the current concentrations against the studied pathogens.

Table 7. Diameters of bacterial growth inhibition rings, estimated in cm, resulting from the extract of thistles of Pterios mils against Klebsella pneumonia

Inhibition diameter (cm)				Concentrations (mg/ml)
Spring 2022	Winter 2022	Fall 2021	Summer 2021	
2.4	3.2	3.5	2.6	raw
1.6	1.3	1.7	2.3	10
1.0	1.2	1.6	2.0	20
1.2	1.4	1.6	1.9	30
1.3	1.3	1.7	1.9	40
1.1	1.1	1.4	1.7	50

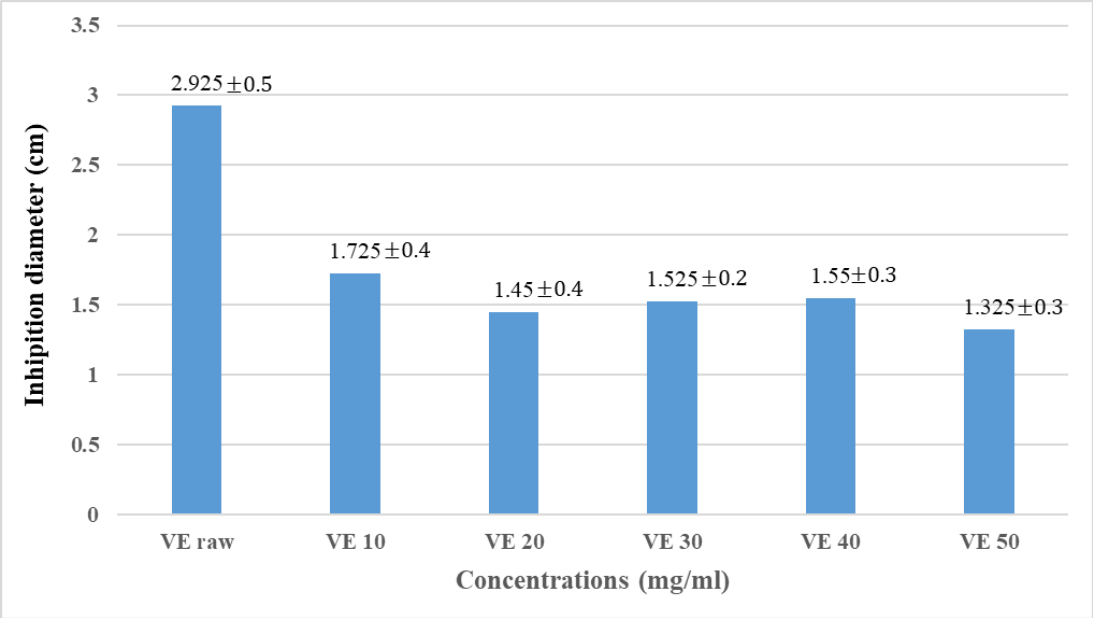

Fig. 4. The mean diameters of bacterial growth inhibition rings, estimated in cm, resulting from the toxic extract Pterios mils of lionfish towards Klebsella pneumonia

Table 8. Comparison of the highest inhibitory concentration of Pterios mils extract with the effect of antibiotics towards the studied pathogenic bacteria

Antibiotics			The concentration adopted in the study						Germs
DOR	TET	CPZ	Pure	10	20	30	40	50	
0.70	1.36	-	2.20	-	-	-	-	-	*Pseudomanas*
0.90	-	2.46	2.92	-	-	-	-	-	Klebsella pneumonia

Table (8) shows a comparison of the highest inhibitory concentration of Pterios mils extract with the effect of antibiotics taken against the studied pathogenic germs.

The extract of lionfish spines (Pterios mils) showed significant activity against bacteria (*Pseudomanas*, Klebsella pneumonia) at the crude concentration, and the effectiveness values were greater than the values of the effectiveness of the antibiotics used against each of the mentioned bacteria.

5. CONCLUSIONS AND SUGGESTIONS

Through this research, the extract of the thorns of the lionfish showed inhibitory activity against the growth of the studied bacteria, but it differed according to the concentrations used.

The best result of the effectiveness of the extract that achieved at the crude concentration, and this is because the amount of active substances in the raw concentration is greater than it is in the rest of the concentrations. In addition, the effectiveness of the toxic extract was better than the effectiveness of the used antibiotics.

Therefore, we recommend the following:

- It is possible to benefit pharmacologically from the toxic extract of the spines of the lionfish caught in our marine waters, and then benefit from it economically through marketing it.
- The possibility of benefiting from the toxic extracts of lionfish spines as natural materials of therapeutic importance and using them as non-traditional antibiotics to treat some human diseases.
- Investing in the results of research in the field of marine biotechnology and benefiting from it by transferring it to the field of laboratory application, similar to the important global research

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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