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Mosquitocidal activity of *Ophiocoma scolopendrina* extracts against *Culex pipiens* and their antimicrobial potential

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ABSTRACT

The current study aimed to study the potential mosquitocidal activities of the crude extract of the brittle star, *Ophiocoma scolopendrina* against the mosquito vector, *Culex pipiens.* Regarding the antimicrobial potential of *O. scolopendrina*, dichloromethane exhibited higher activity than the other solvents; whereas, it could inhibit a wide range of the tested strains with a moderate potential when compared to the positive controls. Larval mortalities exhibited a concentration-dependent pattern. Ethyl acetate, dichloromethane, and ethanol extracts recorded the highest larval mortality percentages with LC_{50} values of (18.345, 20.45 and 50.966 ppm), respectively. Complete larval mortality was recorded when the 3rd instar larvae were treated with 80 ppm for dichloromethane and ethanol extracts. Tested extracts disrupted the emergence of adults, where adult emergence percentages were highly affected by tested extracts, in particular when the LC_{50} concentration of dichloromethane and ethyl acetate extract was applied, it was reduced to (54.88 and 58.47 %), respectively versus100%for the control. Slight to moderate effects on the sex ratio of emerged adults were recorded particularly for LC_{50} treatments. LC_{50} concentrations of ethyl acetate and dichloromethane induced remarkable repellency percentages with about 79.12 and 70.83, respectively. Meanwhile, LC_{95} concentration severely induced repellent activity. Absolute repellent action was reached when the LC_{95} concentrations of dichloromethane, ethanol, and ethyl acetate extracts were applied. The present study paves the way to discover the potential mosquitocidal and antimicrobial activities of *O. scolopendrina* that disrupted some biological aspects of larvae and adults of the mosquito vector *C. pipiens* of public health importance.

INTRODUCTION

The marine environment is interesting and still providing the human beings with valuable applications and services, whether for food (**Zakaria *et al.* 2018; El-Naggar *et al.* 2019**) or as a potential drugs and bioactive compounds (**Ibrahim *et al.* 2017**). The ocean considers a rich source of bioactive compounds or secondary metabolites with potential biomedical properties. Marine organisms represent a diverse reservoir of bioactive substances which could aid in a wide range of diseases treatment (**Metwally *et al.* 2020; El-Naggar *et al.* 2022**). Environmental pressure like competition for space, nutrition and self-defense have led to the production of a diverse array of compounds like secondary metabolites (**Shaban *et al.* 2020**) which used for communication, defense or any other purposes. Marine natural products have attracted the attention to biologists and chemists all over the world for the last few decades due to their recorded antiviral, antimicrobial, antiprotozoal, antifungal, antihelminthic and anticancer activities (**Wang *et al.* 2004; Cui *et al.* 2020**).

Echinoderms have promising activities as a source of bioactive compounds. Among biomedical investigation on echinoderms, brittle stars (*Ophiuroidea*) possess unknown bioactive substances rather than starfish (*Asteroidea*) and sea cucumbers (*Holothuroidea*) related to their therapeutic properties that needs further in-depth investigations. The brittle star (Ophiuroidea) is an aquatic invertebrate characterized by its ability to regenerate its organs (**Czarkwiani *et al.* 2013**). To date, the presence of some bioactive substances such as terpenes, sulfated sterols, carotenoid sulfate, phenylpropanoids and naphthoquinones in brittle star have been proved.

Mosquitoes are the most important group of insects in term of public health importance. Mosquito-borne diseases consider a major problem in almost all tropical and subtropical regions. Currently there are no vaccines for most of these diseases despite several investigations carried out by scientists to combat threats from mosquito-borne diseases. Mosquitoes transmit various diseases, such as malaria, filariasis, dengue, and Japanese encephalitis causing millions of deaths every year (**Benelli 2015; Hasaballah 2015**). Among mosquitoes, *Culex pipiens* complex is considered the most common mosquito vector through urban and sub-urban areas of Africa. *C. pipiens* mainly transmit lymphatic filariasis which responsible for about 51 million individuals' infections globally. Additionally, there are about 1.4 billion people living in areas at risk of infection (**Deshpande *et al.* 2020**). Some synthetic/chemical insecticides are widely used for control of larvae and adults of mosquito populations. However, the harmful effects of chemical pesticides on non-target populations and the development of resistance to these chemicals in mosquitoes along with the recent resurgence of some mosquito-borne diseases have prompted us to explore alternative, simple and sustainable methods for mosquito control (**Hasaballah *et al.* 2021a; 2022**).

Due to the developed microbial resistance to many antibiotic (**Shawky *et al.* 2021**), it is recommended to search for alternatives particularly from natural origin such as marine invertebrates. However, several studies have been performed to evaluate new antimicrobial activities from different sources to defeat microbial pathogenicity (**Attia *et al.* 2021; Shehabeldine *et al.* 2021; Soliman *et al.* 2022**) whether individually or in combination with the routinely prescribed antibiotics. As well, the natural antimicrobial agents have been gotten could applied to treat the human body infections or to combat the microbial infections to the stored foods so, it could be applied as preservatives which protect the food against biodeterioration (**Suleiman *et al.* 2019**).

The objectives of this study were to investigate the antimicrobial potential and the disruptive effect of different tested extracts from the brittle star, *Ophiocoma scolopendrina* on the larval mortality, adult emergence, sex ratio and repellency. Based on obtained data, ethyl acetate and dichloromethane extracts resemble an important source of possibly new compounds with mosquitocidal properties alternative to chemical insecticides.

MATERIALS AND METHODS

1. Sampling, preservation and identification of specimens

The brittle star specimens were collected manually during summer 2021 from Abu Galum Protected Area along the coast of Aqab Gulf, Egypt. The Abu Galum Protected Area was established by the Prime Ministerial Decree no.1511/1992 covering 458 Km^2 named "Managed Resources Area" of which 121 km^2 sea. It is situated on the west side of the Gulf of Aqaba. Abu Galum covers a total area of 458 km^2; among these 121 km^2 is a marine habitat. But the area from the shoreline to the reef slope about 6 km^2 and about 60 Km^2 of which is intertidal zone. It extends about 25 km along the coast, incorporating a semi-continuous fringing reef as well as inland coastal plain and mountain. After sample collection, specimens were immediately washed with sea water and preserved in ice box containing ice cubes and a few pinches of table salt at −20°C until processing. The identification of samples was carefully done on the basis of morphological characteristics according to the following literature; **Campbell (1987), Cherbonnier (1988), Erwin and Picton (1990) and Lieske and Myers (2004)**.

2. Preparation of the crude extracts

The frozen specimens were left to defrost then washed by tap water and chopped into small pieces. 100 g of specimens were put in 500 ml glass beaker and extracted by soaking in 300 ml of different solvents namely; Methanol (MeOH), Dichloromethane (CH_2Cl_2), Ethanol (EtOH), Ethyl acetate ($C_4H_8O_2$), Acetone (C_3H_6O) and Chloroform ($CHCl_3$) and stored in dark at room temperature for 3 days. Extraction was repeated three times until no color was obtained to ensure complete extraction. The mixture was continuously-stirred with the gentle shaking then filtered through a Whatman 542 filter paper and dried at 40 ^oC using a rotary evaporator (**Ballantine *et al.* 1987**).

3. Insecticidal Bioassay

3.1. Mosquito colony

Laboratory reared larvae of the mosquito, *Culex pipiens* complex were collected from the established colony at the insectary of Medical Entomology, Animal House, Faculty of Science, Al-Azhar University, Cairo, Egypt. Larvae were reared in 40-cm-diameter white plastic bowls containing 1000 mL dechlorinated tap water under laboratory conditions of 27 ± 2 ^{o}C, 75 ± 5% relative humidity and 14-10 h light and dark photoperiod. Larvae were daily provided with fish food as a diet. Emerged adults were kept in (30×30×30 cm) wooden cages supplied with 10% sucrose solution daily.

3.2. Larvicidal activity

Extracts based on six different solvents from the brittle star, *Ophiocoma scolopendrina* mentioned previously were tested for their potential larvicidal activity. The larvicidal activity of these extracts was investigated against the early 3rd larval instar of *C. pipiens* mosquito according to the protocol of world health organization (**WHO 2005**). Briefly, twenty-five larvae from the early third instar were picked up and placed in 500 mL plastic cups and treated with different concentrations of methanol (25, 50, 100, 200 and 400 ppm), dichloromethane (5, 10, 20, 40 and 80 ppm), ethanol (10, 20, 40, 80 and 160 ppm), ethyl acetate (5, 10, 20, 40 and 80 ppm), acetone and chloroform (50, 100, 200, 400 and 800 ppm). For each treatment and for the control group, three replicates were used. Mortality was recorded after 24 h post-treatment.

3.3. Adult emergence and sex ratio

After determination of the lethal concentrations (LC_{50} and LC_{95}), new sets of groups of larvae (25 larvae/group) were separately treated with the aforementioned lethal concentrations alongside with the control to assess its effect on adult emergence and sex ratios of the mosquito, *Culex pipiens* adults. Emerged adults were counted and dead pupae were quantified and excluded to accurately calculate adult emergence following the method of **Khazanie (1979)**. Sex ratio was calculated according to the method of **(Shetty *et al.* 2016)**. Results were calculated as Mean ± SE of three replicates.

3.4. Repellency test

The LC_{50} and LC_{95} concentrations that were calculated from the larvicidal bioassay for different tested solvents were directly applied on the ventral surface of pigeon after abdominal feathers removal and left for 10 min. Then, pigeons were placed for 2 h in the standard cages (30×30×30 cm) contain adult females *C. pipiens* starved for 72 h. The protection or repellency period against mosquito bites was calculated based on counting number of fed mosquitoes. Distilled water was used (without solvents) as a negative control while, the commercial DEET 15% from (Johnson Wax Egypt) was applied as a positive control. The treatments were replicated three times in separate cages. Later, number of fed and unfed females were counted and calculated as described by (**Abbott 1925**) as the following: Repellency% = (A%-B%/100-B%) × 100. Where (A) is the percentage of unfed females in treatment and (B) the percentage of unfed females in control.

4. Antimicrobial assessment of Ophiocoma scolopendrina crude extracts

The prepared extracts were tested in vitro for evaluation of their antimicrobial activities against ten indicator strains which distributed as 3 Gram-negative bacteria, 3 Gram-positive bacteria, 2 filamentous fungi and two yeast species. Agar well diffusion assay was applied to fulfill this experiment, a loopful of each indicator strain was regularly spread on the agar surface, using a sterile cork borer to make 6-mm wells in each of which 100 µl of each extract was filled. Thereafter, petri dishes were incubated and checked for inhibition clear zone formation. Bacterial strains were cultivated on Mueller-Hinton agar medium (code; CM0337, Thermo Scientific, Oxoid Microbiology products) in corresponding to gentamycin as a positive control antibacterial, while fungal strains including yeasts and filamentous fungi were cultivated on malt extract agar MEA medium (code; CM0059, Thermo Scientific, Oxoid Microbiology products) in corresponding to ketoconazole as a positive control antifungal (**Suleiman 2020**).

5. Statistical analysis

The larval mortality data was subjected to linear *probit* analysis and one-way Analysis of Variance (ANOVA) to calculate, LC_{50}, LC_{95}, lower and upper confidence limits, Chi-square values and other statistical items using SPSS ver. 25. *P* value was considered significant at <0.05.

RESULTS

1. Identification of collected samples

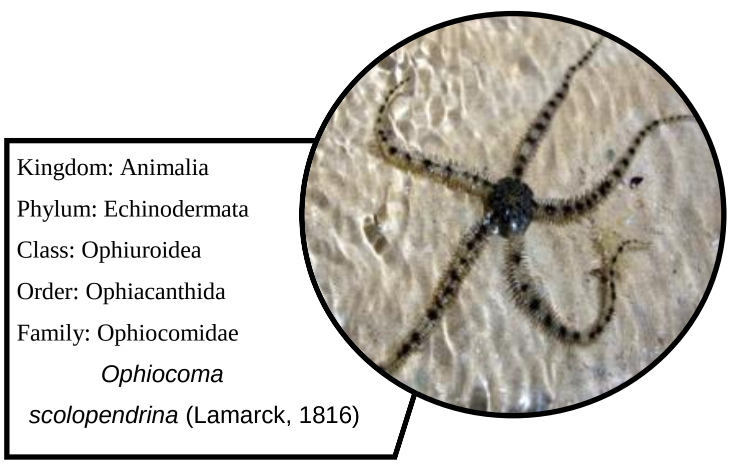

Figure (1). Brittle Star, *Ophiocoma scolopendrina*

The collected brittle star was identified to *Ophiocoma scolopendrina* (Lamarck, 1816) (**Figure** 1). *O. scolopendrina* is a species of brittle star belonging to the family Ophiocomidae. As other brittle stars, *O. scolopendrina* have thin, long arms emerging from a small, disk-shaped body. Dorsally, the disc and arm plates vary from variegated black to pale brown and ventrally, lighter variegated. They are irregularly banded. Its length can be reach to about 13 cm, while the disc diameter can reach up to 25 mm. *O. scolopendrina* occurs in small crevices or under rocks on reef platform and in shallow intertidal area. On inner reef flats, extending its arms from crevices and from under rubble.

2. Toxicity of different solvents used from brittle star against C. pipiens

The newly molted 3rd instar larvae of *C. pipiens* mosquito were treated with six different extracts, obtained with six different solvents, from the brittle star, *O. scolopendrina* namely; methanol, dichloromethane, ethanol, ethyl acetate, acetone and chloroform. **Tables** (1 and 2), revealed that the highest larval mortality (97.3%) was recorded at 80 ppm for larvae treated with ethyl acetate extract. Larval mortality percentages showed a concentration-dependent course.

Table 1. Larvicidal activity of the crude extract of *O. scolopendrina* extracted in methanol, dichloromethane and ethanol against the early 3rd instar larvae of the mosquito, *C. pipiens* 24 h post treatment.

Treatments	Conc. (ppm)	Larval mortality (%) Mean ± SE	Slope ± SE	LC_{50} (LCL–UCL) (ppm)	LC_{95} (LCL–UCL) (ppm)	Statistic summary	χ^2
Methanol	Control	0.0 ± 0.0a	4.69 ± 0.43	96.063 (83.186-111.015)	449.098 (345.867-639.224)	DF= 5, P< 0.001, R^2= 0.939	12.445
	25	12.0 ± 2.31b					
	50	22.67 ± 2.67b					
	100	41.33 ± 3.53c					
	200	81.33 ± 4.81d					
	400	96.0 ± 2.31d					
Dichloromethane	Control	0.0 ± 0.0a	2.98 ± 0.27	20.45 (17.588-23.825)	106.768 (80.311-157.516)	DF= 5, P< 0.001, R^2= 0.949	8.217
	5	13.33 ± 1.33b					
	10	18.67 ± 2.67b					
	20	42.67 ± 1.33c					
	40	77.33 ± 3.53d					
	80	93.33 ± 1.33e					
Ethanol	Control	0.0 ± 0.0a	4.19 ± 0.36	50.966 (44.127-59.266)	241.972 (183.018-354.257)	DF= 5, P< 0.001, R^2= 0.956	7.315
	10	6.67 ± 2.67b					
	20	14.67 ± 1.58b					
	40	37.33 ± 1.17c					
	80	65.33 ± 2.38d					
	160	92.0 ± 2.31e					

Mortalities are presented as Mean ± SE of three replicates, means with different letters are significantly different $P < 0.05$. (LC_{50}) concentration that kills 50% of population, (LC_{95}) concentration that kills 95% of population, (LCL) lower confidence limit, (UCL) upper confidence limit, (χ^2) Chi-square, n= 375.

Table 2. Larvicidal activity of the crude extract of *O. scolopendrina* extracted in ethyl acetate, acetone and chloroform against the 3rd instar larvae of the mosquito, *C. pipiens* 24 h post treatment.

Treatments	Conc. (ppm)	Larval mortality (%) Mean ± SE	Slope ± SE	LC_{50} (LCL–UCL) (ppm)	LC_{95} (LCL–UCL) (ppm)	Statistic summary	χ^2
Ethyl acetate	Control	0.0 ± 0.0a	3.14 ± 0.29	18.345 (15.927-21.121)	81.707 (63.585-114.557)	DF= 5, P< 0.001, R^2= 0.967	7.678
	5	10.67 ± 2.67b					
	10	25.33 ± 1.35c					
	20	45.33 ± 1.17d					
	40	81.33 ± 3.53e					
	80	97.33 ± 1.33f					
Acetone	Control	0.0 ± 0.0a	5.48 ± 0.48	210.66 (181.686-244.657)	1053.01 (799.16-1532.75)	DF= 5, P< 0.001, R^2= 0.972	4.483
	50	8.0 ± 2.31b					
	100	22.67 ± 1.33c					
	200	42.67 ± 1.19d					
	400	78.67 ± 2.52e					
	800	90.67 ± 1.17f					
Chloroform	Control	0.0 ± 0.0a	5.5 ± 0.49	225.781 (194.288-263.071)	1171.92 (880.18-1734.43)	DF= 5, P< 0.001, R^2= 0.977	3.365
	50	5.33 ± 1.26b					
	100	21.33 ± 1.38c					
	200	45.33 ± 2.71d					
	400	76.0 ± 2.3e					
	800	86.77 ± 2.83f					

See footnote Table 1.

Overall, ethyl acetate, dichloromethane and ethanol extracts recorded the highest larval mortality % with LC_{50} values of (18.345, 20.45 and 50.966 ppm) and LC_{95} values of (81.707, 106.768 and 241.972 ppm), respectively. Highest larval mortality was exhibited with highest concentration 80 ppm for dichloromethane and ethanol extracts.

3. Effects of different solvents on the adult emergence and sex ratio percentages

Figure (2) shows that the tested extracts of various solvents played a major role in disrupting the emergence of adults. Adult emergence percentages were highly affected by tested extracts, in particular those treated with the LC_{50} concentrations of dichloromethane and ethyl acetate where it reduced to (54.88 and 58.47 %) versus 100% for the control. This effect was much more pronounced in adults treated as larvae with LC_{95} concentrations. Adult emergence % reduced to 35.35, 43.61, 44.48 and 51.11 when treated with the LC_{95} concentrations of dichloromethane, ethyl acetate, acetone and ethanol extracts, respectively.

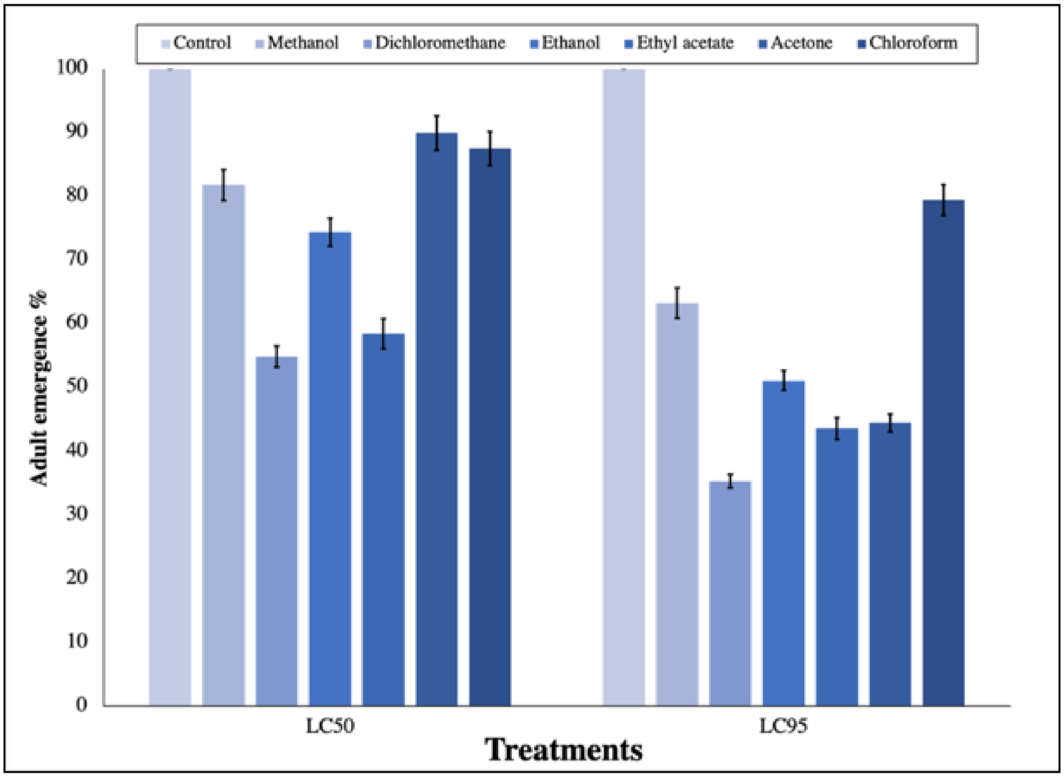

Figure 2. Effect of tested LC_{50} and LC_{95} concentrations of different tested solvents of *Ophiocoma scolopendrina* crude extract on the adult emergence percentages of the mosquito, *Culex pipiens*.

A considerable variation in the sex ratio percentages for those adults treated as larvae with the LC_{50} concentrations was recorded. The percentage of emerged males was higher than emerged female in particular for those treated with dichloromethane, ethanol and ethyl acetate extracts (**Figure** 3a). While for those treated with LC_{95} concentrations, there were no remarkable differences between ratio of both males and females in all tested extracts (**Figure** 3b). Summarizing that larvae treated with LC_{50} concentrations caused significant differences in male to female sex ratio rather than those treated with LC_{95} concentrations.

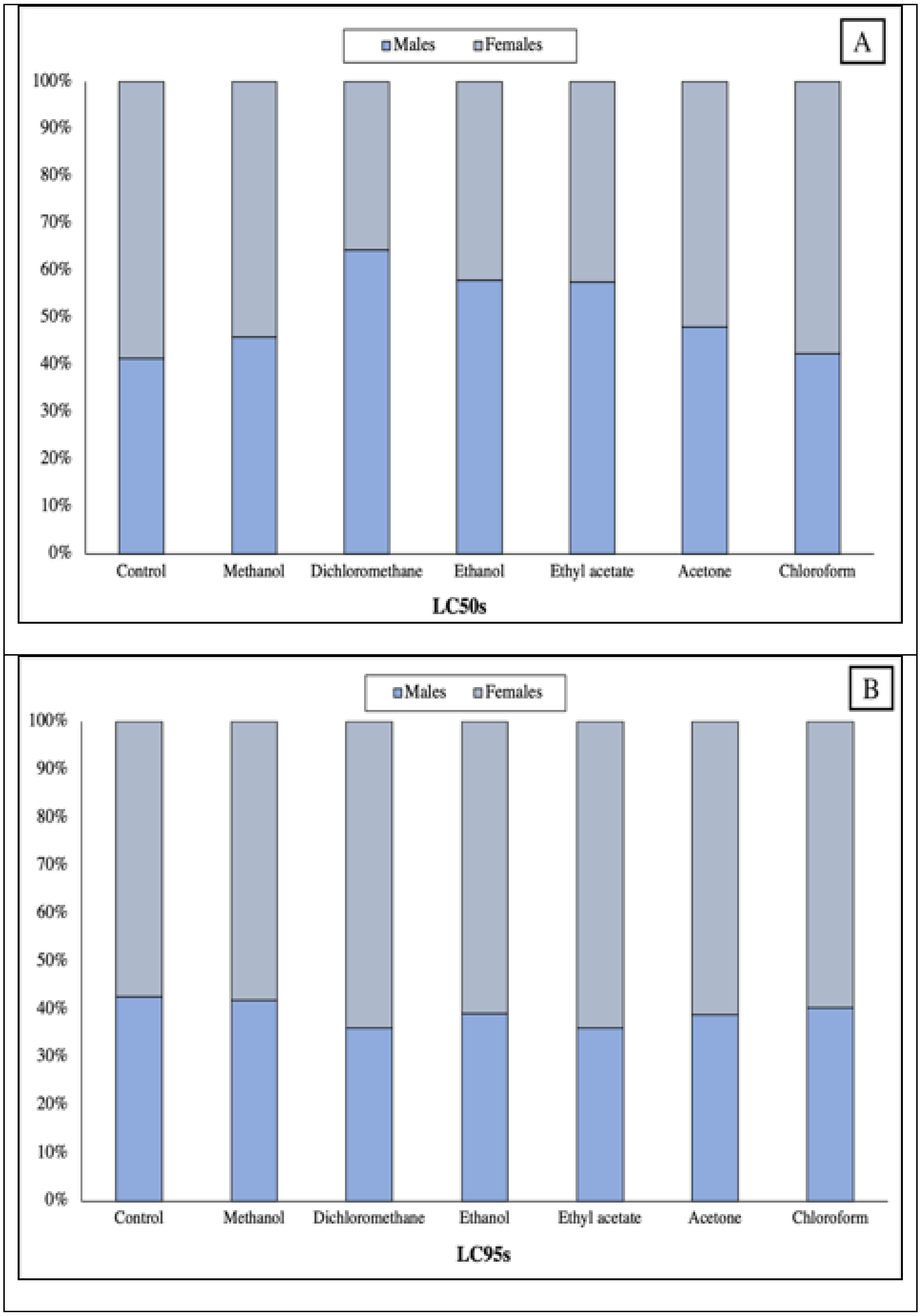

Figure 3. Effect of tested LC_{50} (A) and LC_{95} (B) concentrations of different solvents of *Ophiocoma scolopendrina* crude extract on sex ratio percentages (male to female) of the mosquito, *Culex pipiens*.

4. Repellency effects

Table (3) displays that the efficacy of tested solvents on repelling ability to the mosquito, *C. pipiens* adult females. Repellency effects of tested extracts showed that, at LC_{50} concentrations, ethyl acetate and dichloromethane extracts induced remarkable repellency percentages with about 79.12 and 70.83, respectively. Meanwhile, LC_{95}

concentrations severely induced repellent activity in almost all tested extracts. Complete repellency effect (100%) was reached when LC_{95} concentrations from dichloromethane, ethanol and ethyl acetate extracts were applied compared with 100% of females exposed to the commercial DEET (10 ppm).

Table 3. Repellency effect of tested LC_{50} and LC_{95} concentrations of *O. scolopendrina* solubilized in different solvents against the mosquito, *C. pipiens* adult females.

Treatments	Solvent	Fed females%	Unfed females%	Repellency %
LC_{50}	Methanol	80	20	16.67
	Dichloromethane	28	72	70.83
	Ethanol	36	64	62.5
	Ethyl acetate	20	80	79.12
	Acetone	84	16	12.5
	Chloroform	88	12	8.33
LC_{95}	Methanol	36	64	62.5
	Dichloromethane	0	100	100
	Ethanol	0	100	100
	Ethyl acetate	0	100	100
	Acetone	32	68	66.67
	Chloroform	32	68	66.67
DEET (10 ppm)		0	100	100
Control		96	4	0

5. Evaluation of antimicrobial activity of O. scolopendrina

The antimicrobial activity of the six extracting solvents of *O. scolopendrina* was described in **Table** (4). Dichloromethane extract appears the most potent one among all investigated solvents, dichloromethane extract could affect the growth of 7 indicator strains out of 10 strains. Ethanol exhibited moderate efficacy for extracting the antimicrobial subcomponents, followed by methanol and ethyl acetate. Regarding the indicator strains, one Gram-negative bacterium (*Salmonella typhimurium*), one Gram-positive bacterium (MRSA), and one filamentous fungus (*Aspergillus flavus*) showed complete resistance to all investigated solvents. As well, *Penicillium expansum* showed resistance to all screened solvents except dichloromethane extract. Entirely, all values of inhibition zones ranged from 8 to 20 mm in corresponding to the positive standards which affected the test microorganisms in a range of 15-30 mm. Conclusively, dichloromethane crude extract had a moderate antagonistic effect against a wide range of microbes includes Gram-negative, Gram-positive, yeast, and filamentous fungi.

Table 4. Diameters of inhibition zones resulted by the action of crude extract of *O. scolopendrina* using different solvents.

Test Microorganisms	**Solvents**						**Control**
	Methanol	**Dichloromethane**	**Ethanol**	**Ethyl acetate**	**Acetone**	**Chloroform**	
Escherichia coli **ATCC 25955**	N	12	8	8	N	N	30
Salmonella typhimurium **ATCC 14028**	N	N	N	N	N	N	17
Klebsiella pneumonia **ATCC 13883**	11	12	13	12	11	N	15
Micrococcus **sp. RCMB 028 (1)**	14	13	8	N	N	N	22
Streptococcus mutants **ATCC 25175**	16	18	13	14	16	N	20
Methicillin-resistant S. Aureus **MRSA**	N	N	N	N	N	N	15
Aspergillus flavus **(RCMB 002002)**	N	N	N	N	N	N	16
Cryptococcus neoformans **RCMB 0049001**	N	16	N	N	N	15	25
Candida lipolytica	15	20	14	13	N	16	18
Penicillium expansum **ATCC 25955**	N	12	N	N	N	N	17

N= no inhibition observed.

DISCUSSION

The marine environment is a reservoir of many organisms with biologically active compounds that possess important advantages not found in other organisms. Recent investigations elucidated that marine organism considered one of the most important natural sources and their derivatives/secondary metabolites may possess pesticides, fertilizers, or food sources characteristics for humans and animals alike. These characteristics makes them an important source alternative to many manufactured chemical products. About 7,000 natural marine products have been isolated: 25% from algae, 33% from sponges, 18% from coelenterates (sea whips, sea fans, and soft corals), and 24% from representatives of other invertebrate phyla, such as ascidians, mollusks, echinoderms and bryozoans **(Kijjoa and Sawangwong, 2004; Hasaballah and EL-Naggar, 2017; Cui *et al.* 2020**).

Mosquitoes gain its importance as they transmit diseases that cause morbidity, mortality, economic loss and social disruption (**Milam *et al.* 2000**). Treatment of mosquitoes at the larval stage considered an effective tool for mosquito control. Currently, the use of natural products in mosquito control to prevent the proliferation of mosquito diseases and to protect the environment from the application of chemical insecticides and pesticides gain raised interest. Larval mortality percentages showed a concentration-dependent course with LC_{50} values ranged from (18.345 to 225.781 ppm). At similar rang of lethal concentrations obtained here, **Manilal *et al.* (2009)** reported that the recorded LD_{50} values of the crude extracts of *Lobophora variegate, Spatoglossum asperum* and *Stoechospermum marginatum* were (96.1, 95.5 and 97.3 ppm), respectively against the 3rd instar larvae of *Aedes aegypti*; **Bianco *et al.* (2013)** reported that at 10 ppm hexane extract of red seaweed *Laurencia dendroidea* exhibited complete larvicidal effect (100%) against the larvae of *Ae. aegypti* compared to ethyl acetate (37%), dichloromethane (70%) and methanol (15%). Overall, marine organisms may have a bio-toxic activity against the tested mosquito due to their effects on the hormonal control, by spoiling or destroying the vital organs of its body, or due to the multiple actions of other bioactive compounds present in these organisms.

Herein, different tested extracts played a major role in disrupting the emergence of adults which indicates a disruption of the biological processes of the tested mosquitoes. Adult emergence was reduced to almost the half when larvae were treated with the LC_{50} concentrations of dichloromethane and ethyl acetate and these effects were much more pronounced in adults treated as larvae with the LC_{95} concentrations, these results are in agreement with previous studies, (**Elbanna and Hegazi, 2011**) who reported inhibition/blocking of metabolism, growth and feeding behaviour of mosquito larvae end with death when treated with the crude extract of different types of seaweeds. In insects, adult emergence depends on healthy immature stages. Digestive disorders, starvation, metabolism disturbance, degeneration of peritrophic membranes and accumulation of faecal materials at the hind gut may be due to the active compounds present in the tested extracts marine products.

The Effect of tested extract on sex ratio means that there is a variation in sensitivities of pupae that resulted from the treated larvae which would produce males or females, compared with the pupae from the control group, concluding that toxicological effect on the larval stage may lead to differences in the proportion of males to females. A considerable variation in the sex ratio percentages for adults treated as larvae with the LC_{50} concentrations was recorded while those treated with LC_{95} concentrations exhibited not significant differences between ratio of both males and females in all extracts tested. In the same context, **Gordon (1922)** reported that it possibly that various factors such as food supply influence sex ratios of emerged adults when larvae of *Ae. aegypti* reared on different quantities of foods elucidating more males emerged with maximum food availability than females. **Almehmadi (2010)** reported that 3rd instar larvae treated with LC_{50} of plant extracts *Artemisia herba alba* and *Matricharia chamomilla*, sex ratio was affected in the resulting adult individuals with the number of males greater than the females.

Tested extracts showed severe repellent action for *C. pipiens* adult females that reached 100% when some extracts were applied at LC_{95} concentrations which give promising indication for these extracts as a natural product with repellent properties. These results are in agreement with many previous studies, **Venketachalam and Jebanesan (2001)** who reported that repellent activity of methanol extract of *Ferronia* *elephantum* leaves against *Ae. aegypti* activity at 1.0 mg/cm^2 and 2.5 mg/cm^2 concentrations gave 100% protection up to 2.14 and 4.00 h, respectively and the total percentage of protection was 45.8% at 1.0 mg/cm^2 and 59.0% at 2.5 mg/cm^2 for 10 h. Methanol extract of *Acorus calamus*, *Litsea elliptica* and *Piper aduncum* exhibited LC_{50} values ranging from 0.04 to 0.20 mg/cm^2 towards females of *Ae. aegypti* (**Hidayatulfathi *et al.* 2004**). Essential oil from *Zingiber officinalis* showed repellent activity at 4.0 mg/cm^2 with 100% protection up to 2 h against *C. quinquefasciatus* (**Pushpanathan *et al.* 2008**). Absolute repellency percentage was reached when 10 mg/L of *Sargassum wightii* methanol extract was applied (**Kumar *et al.* 2012**); additionally, *Salix safsaf* leaf extracts showed excellent repellent activity towards the housefly, *Musca domestica* with 76.0% repellency at concentration 800 ppm for petroleum ether and 87.3% protection for ethanol extract (**Hasaballah *et al.* 2021b**).

Based on our knowledge, there are no much information about recruitment of *O. scolopendrina* as a source of antimicrobial metabolites although, the marine atmosphere could offer an exceptional examples of natural products with promising pharmaceutical potential and inspiring therapeutic applications such as the extracts of echinoderms presented a broad spectrum of biological activities whether antimicrobial, anticancer, and anti-inflammatory activities (**Gomes *et al.* 2016**). *Ophioplocus erinaceus* could be considered as source of bioactive substances due to presence of terpenes, sulphated sterols and phenyl propanoides, some of these extracted metabolites exhibited antibacterial activity against microbial strains while other component displayed moderate or high cytotoxicity (**Amini *et al.* 2014**).

CONCLUSION

The results of the current study revealed promising insecticidal and antimicrobial activities of the brittle star, *Ophiocoma scolopendrina* crude extract against the mosquito vector, *Culex pipiens.* Larval mortalities were increased in a concentration-dependent pattern. Ethyl acetate, dichloromethane and ethanol extracts recorded the highest larval mortalities. Tested extracts played a major role in disrupting the emergence of adults with slight to moderate effects on the sex ratios of emerged adults. Additionally, tested extracts exhibited severe repelling activity for LC_{50} treatments and this effect was much more pronounced in the LC_{95} treatments which give an indication for these natural-origin extracts repellent properties. Overall, based on obtained results and besides therapeutic properties, tested extracts and its active constituents can be exploited in integrated pest management programs.

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