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Evaluation Of Antibacterial And Gc-Ms Analysis In The Methanolic Extracts Of Starfish Protoreaster Linckii

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The present study aims to evaluate the antibacterial activity of the methanolic extract of starfish Protoreaster linckii. GC-MS analysis was carried to identify the bioactive compounds responsible for the antibacterial activity. The antibacterial activity of the methanolic extract was tested against five pathogenic bacteria such as Salmonella typhi, Bacillus cereus, Escherichia coli, Vibrio cholerae and Staphylococcus aureus. The extracts were prepared with methanol at three different concentrations 100 µg/ml, 250 µg/ml and 500 µg/ml. The antibacterial activity was measured based on inhibition zone around the well. The result of the study suggested that the methanolic extracts have strong inhibitory activity against different pathogens. Maximum (21±1.52) zone of inhibition was observed at a concentration of 500 µg/ml against gram positive bacteria Staphylococcus aureus and the minimum activity of 2±0.57 against Bacillus cereus at 100 µg/ml concentrations. GC-MS analysis of methanolic extract revealed the presence of 20 bioactive compounds most of which had antibacterial activities. This study proves that the methanolic extract of starfish Protoreaster linckii possess good antibacterial activity.

Keywords : Antibacterial activity, GC-MS analysis, Starfish, Protoreaster linckii, Pathogenic Bacteria.

INTRODUCTION

The marine environment is the world's most productive ecosystem, consisting of abundant faunal and floral diversity [1]. The ocean, which covers more than 70% of earth's surface, is the largest and the most stable ecosystem of all biomes. The world's oceans are widely recognized as the last unexplored territory for human exploration and exploitation of the earth. Further, these vast salty watery regions have inexhaustible resources of food and minerals. The major focus of marine activity tends to lie for food and energy exploration in the benefit of human beings. Discovery of novel products from marine organisms has

inspired scientists all over the world to search for bioactive molecules of medicinal applications [2].

Marine organisms contain numerous organic bioactive compounds including secondary metabolites [3]. These secondary metabolites were used in the agrochemical, cosmeceutical, food, nutraceutical and pharmaceutical industries [4]. Secondary metabolites having desirable antimicrobial properties were isolated from marine organisms [5]. Echinoderms have received great attention as an unexploited source of new bioactive molecules with important antibacterial, antiviral, antiprotozoal, antifungal, anti-helminthic and anticancer activities suggesting their potential applicability for drug discovery [6].

The microorganisms develop multidrug resistance by their peculiar pattern of adaptation behavior and problems of multi-drug resistance in microorganisms are common in every field [7]. The advent of multiple resistance mechanism has severely reduced the use of antibiotics. Therefore the development of new and novel antibacterial drugs to eliminate the disease producing microorganisms is the need of the hour. GC-MS analysis plays an essential role in identifying the active components present in the extract. Hence, the present study was carried out to study the antibacterial activity of starfish Protoreaster linckii and to characterize the bioactive compounds in the methanolic extract of P. linckii by GC-MS analysis.

MATERIALS AND METHODS

Sample Collection and Preparation of Extract

Starfish Protoreaster linckii were from the fishing harbour of Thoothukudi. Collected samples were dried under the shade, then crushed into small pieces, and powdered. The extraction process was carried out by following methodology of Deepak et al., (2020) [8]. The crude extracts were stored at 4ºC and used for further analysis.

Antibacterial assay

The antibacterial activity of the methanolic extracts of starfish Protoreaster linckii was carried out by the well diffusion method proposed by (Reinheimer et al., 1990)[9]. Antibacterial activity was tested against five bacterial strains Bacillus cereus, Salmonella typhi, Staphylococcus aureus, Escherichia coli and Vibrio cholerae were obtained from the Madras Medical College, Chennai. The nutrient broth and agar was prepared and sterilized in an autoclave at 15lbs pressure for 15 minutes. Bacterial strains were inoculated in the nutrient broth at 37° C for 24 hours. Then the pathogens were swabbed on the surface of the Nutrient agar plates. The wells were loaded with various concentrations (100 µg/ml, 250 µg/ml & 500 µg/ml) of methanolic extracts. The plates were incubated at 37ºC for 24 hours. The susceptibility of the test organisms was determined by measuring the diameter of the zone of inhibition. Cefotaxime was used as a positive control. All the extracts were tested in triplicate and the results were expressed as mean ± SD of the three independent values.

GC-MS Analysis

The GC-MS analysis of the methanolic extract of Protoreaster linckii was carried out using Thermo GC-trace standard non-polar column (Dimension: 30 meters, film: 0.25µl). The injector temperature was set at 260^0 C during the chromatographic run. About 1 µl of the

sample was injected into the instrument. The column oven temperature was set at 50^0 C for 3 minutes, raised at 10^0 C per minutes up to 280^0 C and final temperature was increased to 300^0 C for 10 minutes. The ionization voltage was 70 eV. In this chromatography, hydrogen was used as the carrier gas. Column (30m x 0.25mm) was closely fitted to a Perkin Elmer gas chromatography which was equipped with a flame ionization detector. The spectrums of the components were compared with the database of a spectrum of known components stored in the GC-MS NIST (2008) library.

RESULTS

Antibacterial activity of methanolic extracts of starfish Protoreaster linckii was tested against five pathogens and represented in the Figure – 1. The results are expressed with mean and standard deviation. Antibacterial activity was found to be dose-dependent. The different concentration of extracts showed remarkable antibacterial activity against all the pathogens Bacillus cereus, Staphylococcus aureus, Salmonella typhi, Escherichia coli and Vibrio cholera by agar well diffusion method (100 µg/ml, 250 µg/ml and 500 µg/ml). The methanolic extract of P. linckii showed highest activity against Staphylococcus aureus 21±1.52 at 500 µg/ml concentration and 16±0.57 at 250 µg/ml concentration, followed by Salmonella typhi 17±1.52 and Escherichia coli 8±0.57 at 500 µg/ml concentration. The extract showed very weak activity against Bacillus cereus 2±0.57 and Vibrio cholera 3±0.57 at 100 µg/ml concentration. Based on the preliminary screening, starfish P. linckii extracts showed activity against all the pathogens and it can be used as a potential source of antibacterial agent.

GCMS Analysis

GC-MS analysis revealed the presence of twenty bioactive compounds in the extract of starfish Protoreaster linckii. The bioactive compound with their retention time (RT), molecular formula, molecular weight (MW), component area, and activities are present in Table – 1. These compounds could be responsible for various pharmacological activities such as antimicrobial, antioxidant, anticancer, antidiarrheal, anti-inflammatory, anti-proliferative, anti-diabetic, anti-acne, and insecticidal activities.

Figure - 1: Antibacterial activity in the Methanolic Extracts of Protoreaster linckii

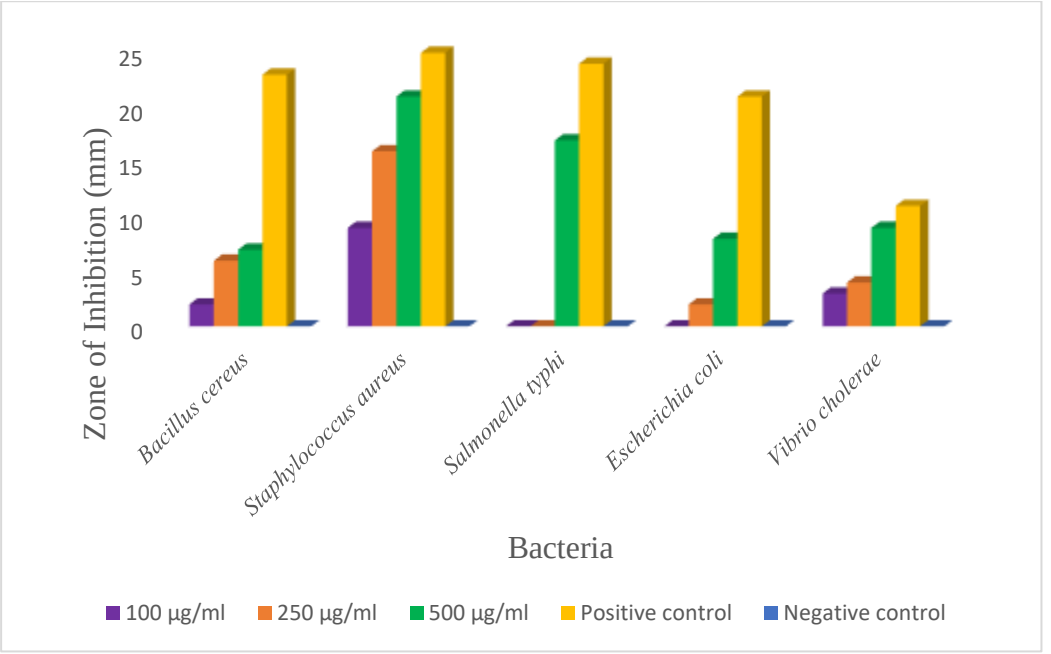

Table 1 Different compounds obtained from methanolic extract of Protoreaster linckii by GC-MS

NO	RT	Compound Name	Molecular Formula	MW	Component area	Match Factor	Activity
1	2.7008	Isobutyl acetate	C6H12O2	116.16	550603320.5	53.9	Antimicrobial
2	3.6100	Propanoic acid, anhydride	C6H10O3	130.14	22894888.0	89.3	Antidiarrheal
3	5.2059	Ethane-1,1-diol dipropanoate	C8H14O4	174.19	37806637.6	83.1	Nil
4	7.7031	3,5-Dimethyl-4-heptanone	C9H18O	142.24	2655617.8	57.7	-
5	12.6378	Naphthalene	C10H8	128.17	1067553022.9	95.2	Antimicrobial
6	14.7242	Butane, 1,1-dibutoxy-	C12H26O2	202.33	10645243.7	95.6	Antimicrobial
7	26.3130	Hexadecanoic acid, methyl ester	C17H34O2	270.5	24091137.3	81.4	Antimicrobial, Anti-cancer, antibacterial,

							antifungal, antioxidant
8	26.8302	n-Hexadecanoic acid	C16H32O2	256.42	112035205.6	96.0	Anti-liver cancer, antimicrobial, antioxidant, cytotoxic
9	27.5935	Heptadecanoic acid	C17H34O2	270.5	18818997.6	72.2	Antioxidant, antidiabetic, anti-proliferative, antimicrobial
10	28.2564	1-Dodecanol	C12H26O	186.33	6560712.1	81.2	Antimicrobial, antioxidant, haemolytic,anti inflammatory, anti-acne, insectisidal
11	29.1851	Octadecanoic acid	C18H36O2	284.5	101710250.5	94.2	Antimicrobial
12	30.2279	O-Arachidonoyl glycidol	C23H36O3	360.5	39374963.6	75.6	Antioxidant
13	30.5957	Octacosanol	C28H58O	410.76	70640216.9	87.5	Nil
14	31.0123	Cis-13-Eicosenoic acid	C20H38O2	310.5	14217922.2	88.9	Nil
15	32.1771	Tetrapentacontane, 1,54-dibromo-	C54H108Br2	917.2	9378073.1	52.5	Nil
16	33.0727	Semioxamazide	C2H5N3O2	103.080	63932865.9	73.2	Antimicrobial
17	33.9629	4-Pentenoic acid, (2,2-diethyl-3-oxo-5-phenyl-, ethyl ester)	C17H22O3	274.35	619336735.7	73.5	Nil
18	34.5908	Tetracosanoic acid, methyl ester	C25H50O2	382.7	21045000.5	57.0	Antioxidant, anticholinesterase

19	35.8919	Ursa-9(11),12-dien-3-one	C30H46O	422.7	24232980.1	61.2	Nil
20	37.0050	Cholest-7-en-14-ol, (5.alpha.)-	C27H46O	386.7	20729183.8	50.5	Nil

DISCUSSION

Marine organisms have been found to produce a great diversity of novel bioactive secondary metabolites and be a potential source for new drug discovery. In the present study, the methanolic extracts of Protoreaster linckii showed considerable antibacterial activity against five pathogens namely Bacillus cereus, Staphylococcus aureus, Salmonella typhi, Escherichia coli and Vibrio cholera. The methanolic extracts of P. linckii showed a maximum zone of inhibition 21±1.52 against Staphylococcus aureus, and 17±1.52 activity against Salmonella typhi at 500µl concentrations and the lower concentration of extracts did not exhibit any zones of inhibition against Escherichia coli and Bacillus cereus.

Mohamed Hussain et al., (2019) [10] carried out antibacterial potential of P. linckii using different extracts such as methanol, acetonitrile, dichloromethane and ethanol at different concentrations ranging from 250 to 1000 µg/ml to study the inhibitory effects of ten selected human urinary tract infectious pathogenic bacteria. Methanolic extracts exhibited a maximum zone (17.00±0.1) of inhibition against gram-positive Staphylococcus saprophyticus at a higher concentration. Minimum zone of inhibition (0.700±0.35 and 0.800±0.15) was reported in gram-positive Staphylococcus aureus using the median and higher concentrations of dichloromethane extracts. But in contrast, the results of the present study shows that the methanolic extracts of P. linckii has highest activity of 21±1.52 against Staphylococcus aureus at 500 µg/ml concentration.

Arockya and Basil (2018) [11] reported the antibacterial activity of various extracts such as butanol, methanol and chloroform derived from digestive glands, tube feet, gonad and spines of the starfish Pentaceraster mammillatus against human pathogenic bacteria Streptococcus mutants, Staphylococcus aureus and Klebsiella pneumonia. In this study the methanolic extract of digestive gland and gonad exhibit activity against Streptococcus mutants (8 mm) and Staphylococcus aureus (10mm). Parajuli et al., (2023) [11] studied the antibacterial potential of Luidia clathrata against both gram positive (Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus, Bacillus cereus and Mycobacterium smegmatis) and gram-negative (Proteus mirabilis, Salmonella typhimurium, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae) bacteria using different extracts like methanol, ethyl acetate and hexane. In this results starfish Luidia clathrata active against all the pathogens. Loredana et al., (2018) [13] investigated the antibacterial activities in three echinoderm species Echinaster sepositus, Arbacia lixula, and Sphaerechinus granularis. The starfish Echinaster sepositus shows activity against Staphylococcus aureus (14±0.5mm), Pseudomonas aeroginosa (7.5±0.2) and Candida famata (8±0.3mm). Present study corroborates with the findings of the above results the methanolic extract of P. linckii was active against Staphylococcus aureus and Escherichia coli bacteria.

In the present study, the presence of antibacterial compounds in the methanolic extract of starfish Protoreaster linckii was characterized by GC-MS analysis. Twenty major compounds were found in the starfish all of these 20 compounds, 8 compounds Isobutyl acetate, Naphthalene, Butane 1 1-dibutoxy-, Octadecanoic acid, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid and Semioxamazide, is responsible for antibacterial properties.

Similarly Mariya et al., (2015) [14] reported the GC-MS analysis of methanolic extracts of Protoreaster linckii. The results revealed the presence of twenty different chemical compounds belonging to broad classes such as saturated fatty acids, unsaturated fatty acids, steroids, carboxylic acids and amides including compounds known to possess potent antimicrobial activity. These compounds were similar to those observed in the present experiment. The present results also shows the starfish P. linckii have twenty compounds most of these compounds possess good antibacterial activity.

Hussan et al., (2020) [15] analysed the four different extracts of star fish Asteropecten spinulosus. The GC-MS analysis of acetone extracts revealed the presence of several bioactive constituents with five major compounds, ethanol extracts revealed seventeen major compounds, ethyl acetate extracts revealed nineteen major compounds and methanol extracts revealed fourteen major compounds. Roman et al., (2019) [16] detected 207 compounds from starfish extracts of Lethasterias fusca by MS. The compounds included 106 asterosaponins, 81 glycosides of polyhydroxyl steroids and 14 polyhydroxylated steroids. These compounds are used for drug development. Based on the results of the present study, starfish Protoreaster linckii has potential antibacterial activity and this trash fish could be used as a lead source in the development of potent antibacterial drugs.

CONCLUSION

Resistance to traditional antibiotics has become a major issue, it is essential to explore natural sources for new antibacterial agents. The marine environment offers a variety of natural bioactive compounds. Marine echinoderms depend on efficient antimicrobial mechanisms for the survival and protection. Star fish are catched along with large quantities of commercially important fishes by trawl. Utilization of this trash into a valuable pharmaceutical product to control pathogenic bacteria may led to the discovery of new antibiotics for the drug resistant bacteria.

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