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Original Article

Antagonistic activity of epiphytic microbes isolated from sea anemones *Anthopleura elegantissima* and *Stichodactyla hadonii from* Kanya kumari coast, against human and fish pathogens

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ABSTRACT

The viable epibiotic microbial community of the marine sea anemones *Stichodactyla hadonii* and *Anthopleura elegantissima* were screened, isolated and assayed for its antagonistic activity against human and fish pathogens. A total of 354 epiphytic bacteria were isolated from the sea anemone *Anthopleura elegantissima*, a relatively high percentage of Gram-positive and non pigmented bacteria (55%) were observed with *Anthopleura* anemone. Whereas most of the *Stichodactyla hadonii* associated strains were gram negative (62%) and non pigmented (84%).7% *A.elegantissima* strains and 12% of *S.hadonii* isolates showed antibacterial activity against fish pathogens and 9% of *A.elegantissima*, 12% of *S.hadonii* isolates showed activity against human pathogens. The inhibition was found to be moderate for most of the tested human and fish pathogens that ranged from 3 mm to 6 mm. Some of the tested pathogens showed trace to no inhibition. *Anthopleura elegantissima* strain 26 produced a maximum zone of 6mm against *Vibrio cholera* and some of the strains of *Stichodactyla hadonii* also inhibited the growth of *V.cholerae*, *E.coli, Pseudomanas aerugenosa* by 4 mm to 5mm also the fish pathogens *Vibrio vulnificus* ,*Vibrio mimicus*, *Aeromonas hydrophila Vibriop harveyi* and *Vibrio parahaemolyticus* were also inhibited by the sea anemone extracts.The efficient antagonistic potential exhibited by the associated microbes from *Stichodactyla hadonii* and *Anthopleura elegantissima* against pathogens may further reflect the potential use of these strains in managing the human and fish diseases that require wide research.

Keywords: Sea anemones-*Anthopleura elegantissima*, *Stichodactyla hadonii*,Epibacterial isolation, Antagonistic activity, Human and Fish pathogens

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INTRODUCTION

The marine environment is an exceptional reservoir of natural products, many of which exhibit structural features, not found in terrestrial natural products. Marine invertebrates, especially sedentary sea anemones are evolved with rich sources of bioactive metabolites, which could be used for novel antimicrobial drugs [1,2]. Sea anemone shows a very good symbiotic partnership with the marine ornamental fishes, especially with clowns. Some of these fish species possess considerable resistance to the sea anemone but appear to be mainly protected by a mucous coat which prevents discharge of the nematocyst [3].Marine invertebrates have developed highly specific relationships with numerous associated microorganisms and these associations are of recognized as ecological and biological importance [4,5 and 6]. It has been reported that the ratio of microorganisms with antimicrobial activity from invertebrates was higher than from other sources [7,8] which suggests that invertebrate-associated microorganisms might play a chemical defence role for their hosts. This kind of microorganism as a sustainable resource has a high potential to biosynthesize novel biologically active secondary metabolites.

In particular, novel secondary metabolites, including antibiotics from marine bacteria are attracting attention because of the growing demand for new antibiotics [9]. Marine microorganisms are of considerable current interest as a new and promising source of biologically active compounds. They produce a variety of metabolites, some of which can be used for drug development [10, 11,12, 13 and 14]. Recently, it was shown that some bioactive compounds isolated from invertebrates originate from symbiotic microorganisms. There is a copious number of works pertaining to the antibacterial agents from marine bacteria, microalgae, seaweeds, sponges, molluscs and ascidians [15,16].These organisms can attach to living and non-living surfaces like medical devices which include urinary,venous, and arterial catheters, shunts, heart valves and tubes [17]. According to a publication by the National Institutes of Health, more than 80% of all infections involve biofilms [18,19].

Association between marine invertebrates and symbiotic bacteria are increasingly recognized as widespread and of biological importance [20].However, to date, the biodiversity of marine microbes and the versatility of their bioactive metabolites have not been fully explored. Production of bacteriostatic substances by marine bacteria has been amply documented during the last few years [21] Sea anemones are evolved with rich sources of bioactive metabolites, which could be used for novel antimicrobial drugs, many of which exhibit structural features, not found in terrestrial natural products. Some natural products were extracted from marine organisms, but less than 1% has been examined so far for pharmacological activity[22].Sea anemones, like other coelenterates, produce many biologically active polypeptides and proteins, including neurotoxins, pore-forming toxins (or cytolysins), phospholipases and proteinase inhibitors [23,24]. Therefore, they have evolved the ability to synthesize toxic compounds obtained from marine microorganisms. These compounds help them deter predators, keep a competitor at bay or paralyze their prey [25]. Sea anemone shows a very good symbiotic partnership with the marine ornamental fishes, especially with clowns. Some of these fish species possess considerable resistance to the sea anemone but appear to be mainly protected by a mucous coat which prevents discharge of the nematocyst [26,27 and 28].

As a consequence of increasing demand for the biodiversity in the screening programs seeking therapeutic drugs from natural products, there is now a greater interest in marine organism. There is a copious number of works pertaining to the antibacterial agents from marine bacteria, microalgae, seaweeds, sponges, molluscs and ascidians [15,16].There is no much work on the epibiotic bacterial isolation and its beneficial activity from the sea anemones. So in this study an attempt has been made to assess the antagonistic activity of the epiphytic microbes isolated from the sea anemones *Stichodactyla haddoni* and *Anthopleura* against the human and fish pathogens.

MATERIALS AND METHODS

The sea anemones *Anthopleura elegantissima* and *Stichodactyla hadonii* for the present study were collected from Kanyakumari coastal area. The Kanyakumari coast (Lat. N08^{o} 04’463 Long. E77^{o}31’ 270 – Lat. N08^{o}04’403 Long. E77^{o}33’075) is located at the southern tip of Tamil Nadu state, India and is the meeting point of Arabian Sea, Bay of Bengal and the Indian Ocean. The sea anemones were collected from the Kanyakumari coast in a sterile polythene bags and brought immediately to the laboratory for the isolation of epibiotic bacteria. The anemones were washed with sterile sea water to remove the debris and salt particles and also the non attached bacterial flora following the method [29].

Epibacterial isolation: Viable heterotrophic bacteria were obtained swabbing a small area ($1cm^2$, in three replicates) of each specimen’s external surface with a sterile cotton-swab, which were then placed in 2 ml of sterile seawater and vortexed. Serial 10-fold dilutions of each solution were prepared and aliquots (0.1 ml) were plated on Marine Agar 2216 (MA, Difco) and Thiosulphate Citrate Bile Sucrose (TCBS, Difco) Agar in replicates following the method of [30]. Plates were incubated for 7–10 days at 20^0 C. Colony-forming units (CFU) were counted and noted; number of pigmented and non pigmented strains was noted. Perceptible different morpho types were isolated in pure culture on MA plates. Bacterial isolates were kept in slant cultures at 4 ^{0}C. Gram staining procedure was followed for all the strains.

Antibacterial activity of the Isolated epibiotic bacteria against Human and Fish Pathogens: Antagonistic effect against 10 human pathogens such as *Escherichia coli* (ATCC 25922), *Shigella dysentriae* (ATCC 13313), *Staphylococcus epidermidis* (ATCC 12228), *S. aureus* (ATCC 29737), *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas aerogenosa* (ATCC 10197), *Vibrio cholerae* (ATCC 14100*)*, *Streptococcus pneumoniae* (10015), *S. faecalis* (10741), *Bacillus cereus* (ATCC 10876)] and 8 fish pathogens such as *Serratia marcescens* (MTCC 97), *Proteus mirabilis* (MTCC 1429), *Aeromonas hydrophila* (ATCC 7966), *Micrococcus* sp.(Fish isolate), *V. vulnificus* (ATCC 27562), *V.parahaemolyticus* (ATTC 17803), *V. harveyi*, (shrimp isolate) and *V. mimicus* were used as test strains. The test strains were obtained from Vellore Christian Medical College. All the test organisms were cultured in Tryptone soya broth (TSB) and the 18-24 hours old broth were used for the experiments.

The method described by [6] was followed for antibacterial assay with modification of the expression of results in percentage of inhibition. Three distinct epibacterial genera were individually applied as a single streak on the Zobel Marine Agar (ZMA Hi media,Mumbai) plate and incubated at 28^0 C for 48 hours. Then the human and fish pathogens were applied as a single streak perpendicular to the epibacterial streak without touching the epibacterial strain streak and the plates were incubated for another 24 hours and the inhibition zones were compared and noted Individual control plates without epibacterial streaks were maintained separately.

RESULTS

Sea anemone Collection and Identification: In the present study the sea anemones *Stichodactyla hadonii* and *Anthopleura elegantissima* the marine sea anemones that is widely distributed along the rocky tidal areas of kanyakumari coast which is the meeting point of Arabian Sea, Bay of Bengal and the Indian Ocean was collected with help of SCUBA divers and identified using the standard procedures [31] (Figure.1 and 2).

Fig.1.*Stichodactyla hadonii*

Fig.2.*Anthopleura elegantissima*

Epibacterial isolation: The microorganisms associated with sea anemones were isolated in order to assess the potential of these microorganisms for the production of antimicrobial compounds. The isolates were designated according to the names of sea anemones as SH and AE. All the isolates were subjected for antimicrobial activity against 10 human and 8 fish pathogens. A total of 354 strains from *Anthopleura elegantissima* and 164 strains from *Stichodactyla hadonii* were isolated.

Antibacterial activity of the isolated epibiotic bacteria against Human and Fish Pathogens: Countable number of bacterial load was present in both the Sea anemones. Out of the 354 strains isolated from *A. elegantissima,* 294 strains were non pigmented and 60 strains were pigmented and mucoidal in nature. The percentage of gram positive strains were slightly dominant over the gram negative strains, i.e. is 55% (160) of the isolated strains were gram positive and 45% (194) of the strains were gram negative (Fig.3).Majority of the gram negative strains exhibited inhibitory activity against most of the human pathogens and 79% showed activity against fish pathogens, whereas 21% (5) of the gram positive strains exhibited inhibitory activity against fish pathogens and 19% showed antibacterial activity against human pathogens (Fig.4).

A total of 164 bacterial strains were isolated from *S. hadonii* and were recorded based on their colony morphology and pigmentations. Among the 164 strains isolated, 138 (84%) were found to be non pigmented and 26 (16%) were pigmented and mucoidal colonies.62% of the strains were identified as gram negative and 38% were identified as gram positive. Here 30% of the gram positive strains exhibited good antibacterial activity against the fish pathogens and 17% exhibited activity against human pathogens (Fig. 5 and 6).

Tables 1 show the inhibitory effect of the epiphytic bacteria isolated from *S. hadonii* against human pathogens. It was noted that the strains of SH 4, 22, 31, 17, 47, 94, 96, 98 and 103 inhibited the growth of *V.cholerae* to 4mm, followed by the strains SH 46, 96,103 which also inhibited *E.coli* to 4mm. The strains SH 37,46,89,94 and 96 exhibited an antibacterial activity of 4 mm against *Pseudomonas mirabilis*. Inhibitory activity of 5mm was observed for the pathogen *B.cereus* and *S.epidermidis* by the strains SH 47, 60, 89, SH 60 and 94, also the strains SH 22, 46, 47, 60, 89, 103,114 and 162 inhibited the growth of *Salmonella typhi* and *Pseudomonas aeurogenosa* by 4mm.The inhibition was found to be moderate for all the tested pathogens (Fig.7)

In the case of fish pathogens, the epibacterial strains SH 32,109 and 164, exhibited a moderate inhibitory activity of 3 to 4mm against *Vibrio vulnificus* and 3 to 5 mm against *Vibrio mimicus.* The strain SH 13,109,164 produced a bactericidal effect of 4 to 5mm against *Aeromonas hydrophila* and *Vibriop harveyi*. *Vibrio parahaemolyticus* showed an inhibitory zone of 6mm against the epibacterial strain SH 109 and 5mm against SHI64 (Fig.7). Most of the *S.hadonii* epibacterial strains tested against the fish pathogens showed good antagonistic activity compared to the human pathogens. Gram-negative strains were found to be the producers while comparing the results with the gram-positive strains (Table 2).

Tables 3 show the inhibitory activity of the epibacterial genera isolated from the sea anemone *Anthopleura elegantissima* against human pathogens .The strains AE 55 and 342 showed an inhibitory zone of 5mm against *P.mirabilis.* The strains AE 11, 55, 96 and 206 are good enough to inhibit the growth *of P.mirabilis* to 4mm.The strain AE 55 inhibited, *Salmonella typhi, Bacillus cereus* to 5mm and *E.aerugenes* to 4mm. While the strains.AE 32,316 and 342 exhibited a maximum inhibitory zone of 5 mm against *Salmonella typhi* and *k.pneumoniae.* The strain AE 26 is found to be the more potent strain by producing an activity of 6mm against *Vibrio cholera*. Most of the AE strains tested showed moderate inhibitory activities against the human pathogens tested. Gram-negative strains were found to be more producers than the positive strains. The results of the antibacterial activity of *Anthopleura elegantissima* epiphytic strains against fish pathogens showed that out of the total 354 strains the Strain (AE 263) inhibited the fish pathogens *A. hydrophyla* with a zone size of, 5mm and the strains AE 31, 42, 54, 63, 67, 263, 274,306 and 351 exhibited an antibacterial activity of 4mm against *A. hydrophyla* and *P.mirabilis*. AE 42 and 274 inhibited *Vibrio parahaemolyticus* to a maximum of 5mm (Fig 8; Table 4).

Table 1. *Stichodactyla hadonii* associated bacteria exhibiting antagonistic activity against human pathogens

Human Pathogens										
Isolated Strains	*E.coli*	*P.mirablis*	*S.epidermidis*	*E.aerugenes*	*K.pneumoniae*	*P.aerogenosa*	*V.cholerae*	*S.pneumoniae*	*S.typhi*	*B.cereus*
	Zone of inhibitions(mm)									
SH1	2	1	T	2	T	T	T	T	-	T
SH4	1	2	2	1	T	2	4	3	2	4
SH12	T	-	-	T	T	1	2	1	T	3
SH22	2	2	3	2	1	T	4	5	4	2
SH31	3	3	3	4	4	3	4	6	2	3
SH32	T	-	2	1	T	-	-	T	1	T
SH37	3	4	3	4	3	4	4	3	2	3

SH46	4	4	4	2	2	3	2	3	4	3
SH47	3	2	4	4	4	4	4	5	4	5
SH60	6	6	5	5	5	4	6	5	5	5
SH62	T	-	-	T	1	2	T	-	1	T
SH76	-	-	T	T	1	2	-	T	1	-
SH78	4	3	2	2	1	3	5	4	3	2
SH80	T	-	-	-	T	1	2	T	2	T
SH86	-	T	T	T	T	T	2	-	-	T
SH89	2	4	2	2	2	3	5	4	4	5
SH91	1	T	2	3	T		-		T	1
SH94	3	4	5	4	3	2	4	4	3	4
SH96	4	4	3	2	-	3	4	4	5	4
SH98	3	4	T	3	2	3	4	1	2	3
SH103	4	3	2	4	4	4	4	3	4	4
SH114	3	2	2	2	2	1	3	3	4	4
SH162	3	2	3	4	3	3	T	1	2	2
SH 164	2	-	3	T	T	T	T	T	T	T

SH - *Stichodactyla hadonii* ; T -Trace

Table 2. *Stichodactyla hadonii* associated bacteria exhibiting antagonistic activity against fish pathogens

Isolated Strains	*Fish Pathogens*							
	V.vulnificus	*V.mimicus*	*V.harveyi*	*Aeromonas hydrophyla*	*Proteus mirabilis*	*Micrococcus sp.*	*S.marcescense*	*V.parahaemolyticus*
	Zone of inhibitions							
SH2	-	-	-	T	1	-	-	1
SH7		1	-	T	-	-	T	1
SH13	4	2	-	4	3	3	-	4
SH15	2	2	T	-	T	T	T	3
SH21	2	2	T	T	T	-	2	T
SH32	3	4	5	4	3	1	3	2
SH37	1	2	T	T	3	2	1	2
SH45	2	3	T	T	-	T	-	3
SH56	T	2	3	2	T	T	T	2
SH62	2	2	4	2	2	1	T	-
SH78	-	T	T	2	1	2	1	2
SH96	T	2	T	T	-	2	2	T
SH98	T	-	-	-	-	-	T	-
SH103	-	2	T	1	3	T	-	T
SH109	4	5	3	4	2	3	3	6
SH114	3	T	-	3	3	2	-	T
SH152	T	-	-	-	-	-	T	T
SH162	T	1	1	2	2	3	T	T
SH164	4	3	4	5	3	3	4	5

SH - *Stichodactyla hadonii*, - *No activity*, *T- Trace*

Table. 3. *Anthopleura elegantissima* associated bacteria exhibiting antagonistic activity against human pathogens

	Human Pathogens									
Isolated Strains	*E.coli*	*P.mirablis*	*S.epidermidis*	*E.aerugenes*	*K.pneumoniae*	*P.aerogenosa*	*V.cholerae*	*S.pneumoniae*	*S.typhi*	*B.cereus*
	Zone of inhibitions									
AE5	2	1	-	1	-	T	T	-	-	-
AE9	2	1	2	2	T	3	2	2	T	1
AE11	3	4	3	2	5	4	3	4	3	3
AE15	T	-	1	2	T	-	1	2	1	1
AE19	1	2	T	1	4	4	1	2	3	1
AE22	2	1	-	-	1	T	-	-	2	1
AE23	1	3	2	1	T	-	T	4	2	1
AE26	2	1	1	4	4	2	6	4	5	2
AE28	1	T	T	1	1	T	T	T	-	T
AE32	1	T	-	-	4	3	2	T	1	1
AE33	1	2	T	-	1	1	2	3	T	1
AE42	2	2	1	1	T	T	2	2	2	2
AE47	2	1	2	3	T	T	1	2	T	1
AE50	2	1	-	-	-	-	-	-	-	T
AE55	3	4	5	6	3	5	2	1	4	5
AE72	2	T	T	-	-	-		-	-	-
AE74	3	3	-	T	-	4	2	1	T	T
AE96	3	4	5	4	3	5	2	4	1	1
AE103	1	2	3	3	1	1	T	T	-	T
AE118	1	2	3	3	1	2	2	1	1	1
AE121	-	T	2	3	2	T	-	-	1	2
AE129	T	1	1	1	T	T	T	T	T	T
AE134	2	3	4	3	1	1	T	T	1	1
AE138	2	1	1	-	-	T	T	-	2	2
AE206	3	4	4	3	4	5	4	3	2	2
AE212	T	T	1	-	1	-	-	T	T	-
AE256	-	-	-	-	2	4	T	1	1	1
AE302	T	-	-	3	-	-	2	1	2	1
AE312	2	1	1	1	2	3	2	1	1	T
AE316	T	-	1	3	5	2	1	1	5	1
AE342	1	5	2	2	5	2	1	3	5	1

AE- *Anthopleura elegantissima*
T-Trace, (-) : No activity

Table 4. *Anthopleura elegantissima* associated bacteria exhibiting antagonistic activity against fish pathogens

Isolated Strains	*Fish Pathogens*							
	V.vulnificus	*V.mimicus*	*V.harveyi*	*Aeromonas hydrophyla*	*Proteus mirabilis*	*Micrococcus sp.*	*S.marcescense*	*V.parahaemolyticus*
	Zone of inhibitions							
AE18	1	-	-	T	1	2	-	1
AE26	2	1	-	T	-	-	T	1
AE31	4	3	4	4	3	3	3	4
AE42	3	2	3	3	4	4	3	5
AE48	2	3	2	3	1	3	2	3
AE54	3	2	3	4	3	3	2	4
AE63	4	4	3	3	2	4	3	3
AE115	3	2	3	2	3	3	2	1
AE167	3	2	2	4	4	2	4	3
AE192	3	3	2	2	3	2	-	3
AE199	-	-	T	1	T	T	-	1
AE205	-	-	-	-	-	1	T	-
AE212	1	2	2	T	-	-	-	-
AE235	2	2	3	3	T	-	T	2
AE263	4	5	5	4	4	4	5	4
AE274	5	4	6	5	5	4	4	5
AE292	-	T	1	T	-	-	T	T
AE301	T		T	-	-	1	-	1
AE306	3	4	4	4	3	5	4	5
AE314	2	1	1	2	2	3	2	2
AE326	T	1	-	-T	-	1	2	1
AE341	2	3	3	2	2	1	3	2
AE351	3	3	4	4	3	3	3	3
AE352	5	T	5	T	T	T	4	4

AE- *Anthopleura elegantisiima, - No activity, T- Trace*

DISCUSSION

Bacteria associated with marine invertebrates have been shown to have significant bioactivity including antifouling, antibacterial and cytotoxic activities and there have already been numerous studies on marine medical exploration and ecology investigation about sponges associated bacteria and fungi [32,33 and 34]. In the present study out of the 354 strains isolated from the sea anemone *A.elegantissima* majority of the strains were gram positive (55%) and non pigmented (294) where as in the case of *S.hadonii* the percentage of gram negative strains slightly dominated (62%) and also a higher percentage (84%) of non pigmented strains were also encountered. Most of the gram negative strains showed antimicrobial activity against the tested human and fish pathogens and this is in support by the work of [35] who stated that 28% (8/29) of the total bacterial strains isolated from the marine sponge *Hymeniacidon perleve* were gram negative.This observation is also on par with the findings of [36]. The finding of the present study that the percentage of Gram positive strains lower (38%) than the Gram negative strains (62%) of *S.hadonii* agrees with one of the earlier works of [37]who had observed that out of the 352 bacterial strains isolated from the gorgonid corals, *Subergorgia suberosa* and *Junceella juncea*, 61% were identified as Gram-negative. Another study revealed that the bacterial strains isolated from various regimens of the marine environ showed that 82.28% were Gram-negative [6].This work was also supported by the result of [34] that 17 % of the pigmented strains showed activity against the pathogens tested. In the present study a smaller percentage of the pigmented strains also produced activity which is substantiated by the earlier findings of [6]. The percentage of baterial isolates of sea anemones antagonistic to human and fish pathogenic bacteria was found to be 7% (*A.elegantissima*) and 12% (*S.hadonii*) against fish pathogens and 9% (*A.elegantissima*) and 12% (*S.hadonii)* against human pathogens. [38] Isolated the bacterial strains from different biotic and abiotic surfaces of the marine environment and found out that, out of 170 isolates screened, 101 proved active against fish pathogens. Also [39] had opined the control of bacterial pathogens associated with fish diseases by antagonistic marine actinomycetes isolated from marine sediments. The inhibitory activity of 6 mm by AE 26 against *V.cholera* and 5 mm against *Salmonella typhi* and *K .pneumoniae* (AE 32,316 and 342), 4 to 5mm by the strains SH4, 22,31 ,37, 17, 46, 47, 89, 94, 96,98,103 against *V.cholerae E.coli* and *Pseudomonas mirabilis* collides with the result of [36] who had carried out similar works on the bacteria associated with marine organisms and the authors have reported that *Alteromonas* strains isolated from sea plants exhibited antagonism against all the test organisms (*Listeria monocytogens*,*Salmonella typhimurium* and *E.coli*).The data obtained from antimicrobial activity of epiphytic bacteria from *S.haddoni* and *A.elegantissima* against the fish pathogens like *Aeromonas hydrophila*, *Vibrio harveyi* and *Vibrio parahaemolyticus* with the inhibitory zone of 4 to 6 mm against the epibacterial strain AE263, *S*H13,109,164 SH 109 and SH I6 also merges with the result of [40], [26] who screened and isolated the antibiotic compounds from the mucus of sea anemone *Heteractis magnifica*, and evaluated the antimicrobial activity of epiphytes against fish pathogens. The antimicrobial activity of the toxin produced by *H. magnifica* and *S. meritensis* and identified that some extracts showed highest inhibition against *S. aureus* (69.23%) and *S. typhi* (63.16%)[25].

Although there were many studies on marine medical screening and ecological investigation pertaining to sponge, sea weed associated bacteria and fungi [41] there is scarcity of report about the epiphytic bacteria with antimicrobial activity of sea anemones. The results implied that some marine animal associated bacteria could probably release various antibiotic compounds to provide themselves the survival competition superiority. Marine microorganisms as model systems offer the potential to understand and develop treatments for disease based on the normal physiological role of their secondary metabolites [10] and are currently being applied to the development of new drugs. In order to find more novel structures, new ways of screening of these compounds should be applied.

CONCLUSIONS

Thus in the present study the recovery of strains with antibacterial activity suggests that sea anemones are ecological niche animals which harbours a largely uncharacterized microbial diversity and a yet unexplored potential in the search for new secondary metabolites. The antagonistic marine bacteria isolated from the sea anemones may produce antibacterial potential compounds which can be explored to generate pronounced biological activity in the future. Isolation and purification of the constituent active compounds is necessary in order to identify their chemical nature and to evaluate their potential as novel drugs which can be used in the development of new antifouling compounds as well as novel antimicrobial agents for pharmaceutical development.

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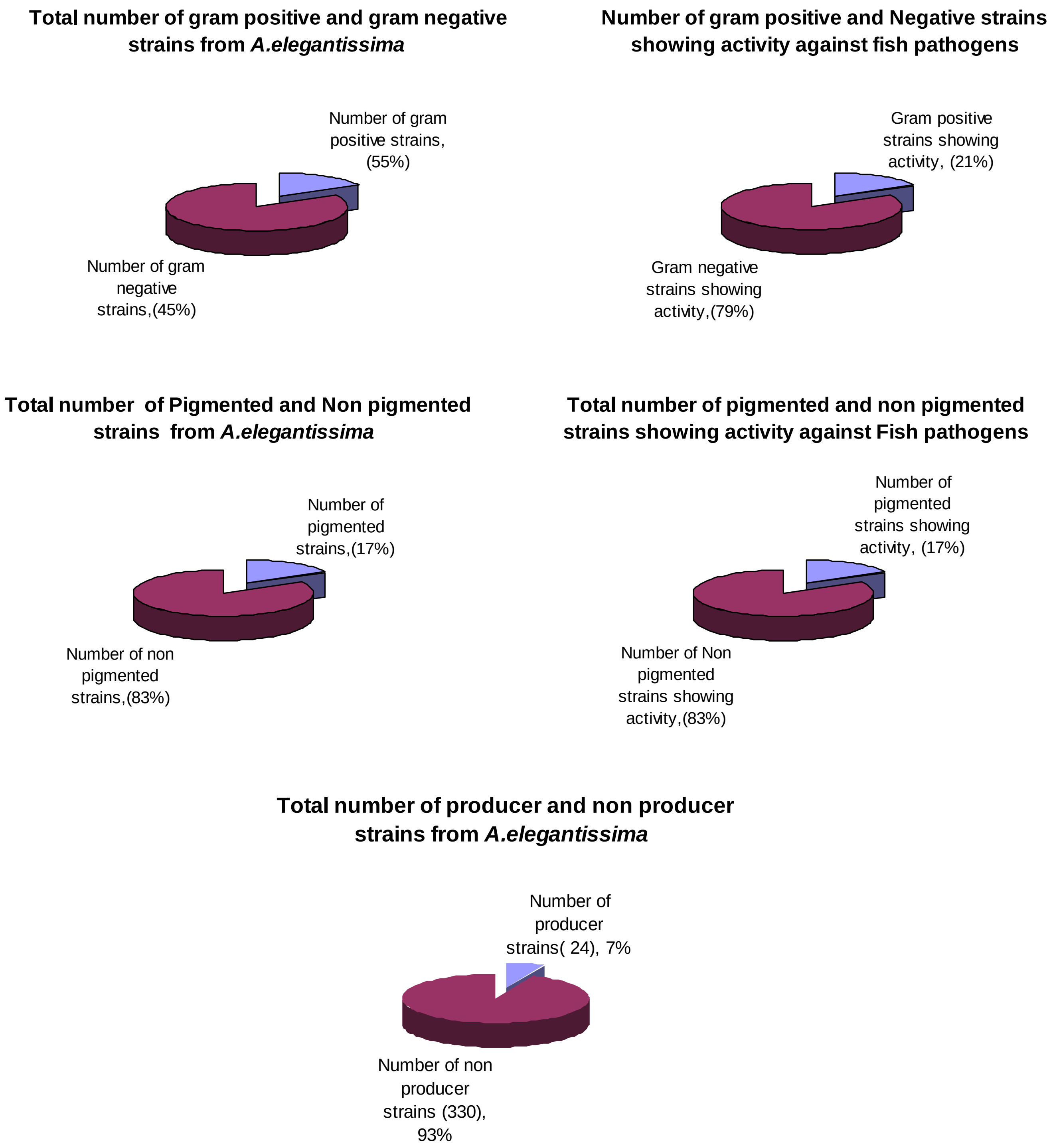


Fig.3.The Isolated Microbes from *A.elegantissima* showing Activity against fish pathogens.

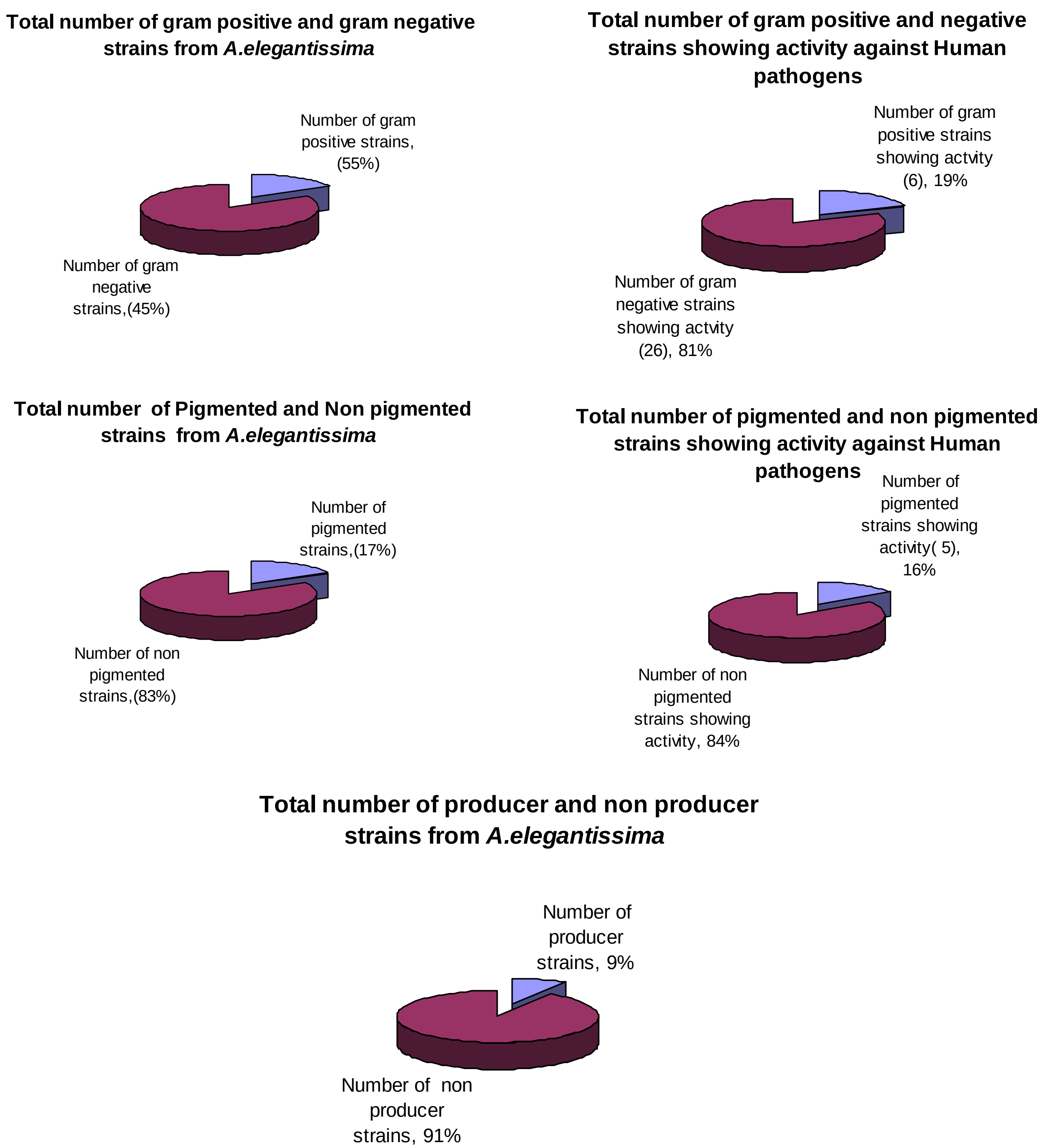


Fig. 4. The Isolated Microbes from *A.elegantissima* showing Activity against Human Pathogens

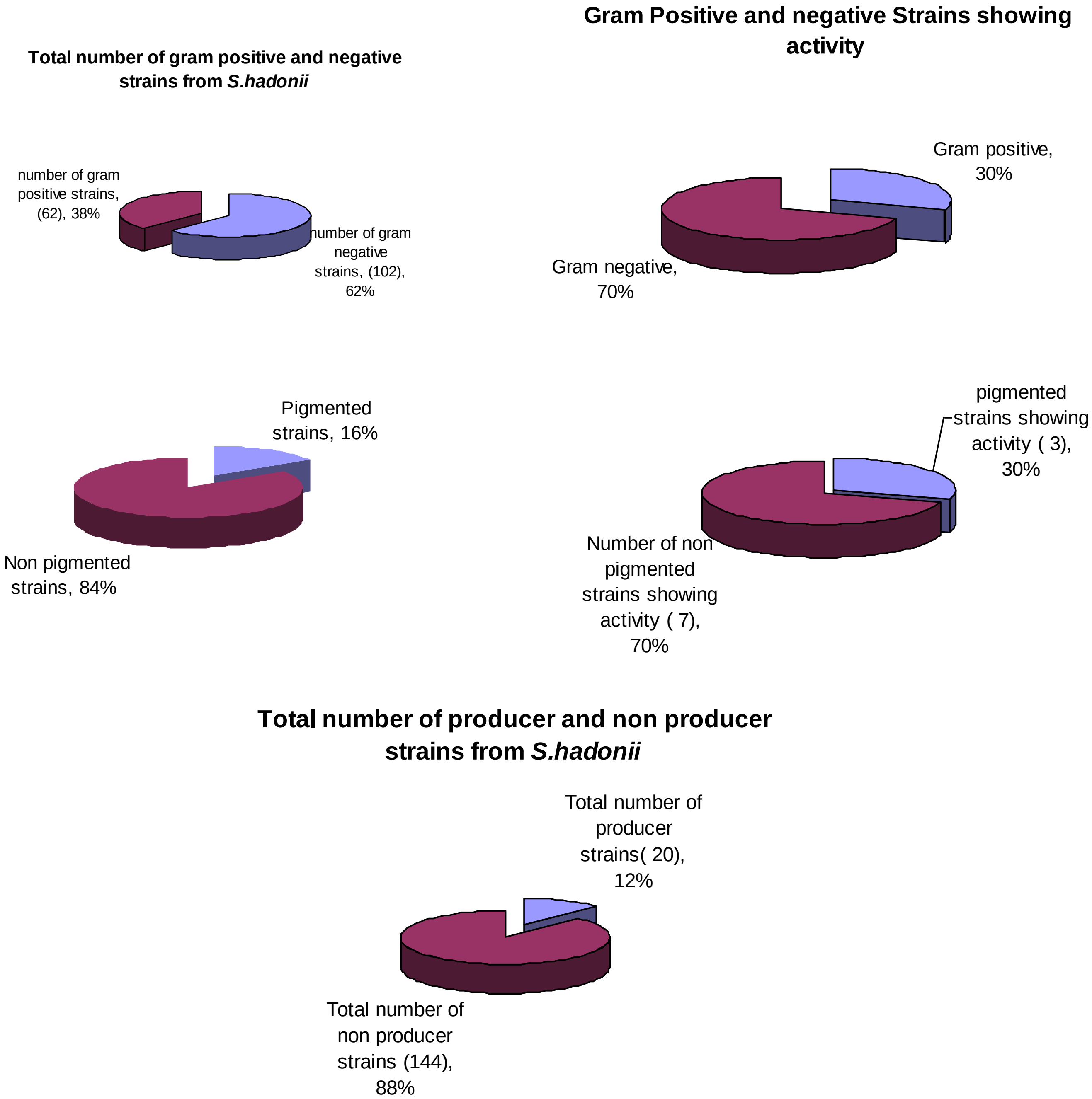


Fig. 5. The isolated microbes from *S.hadonii* showing activity against fish pathogens

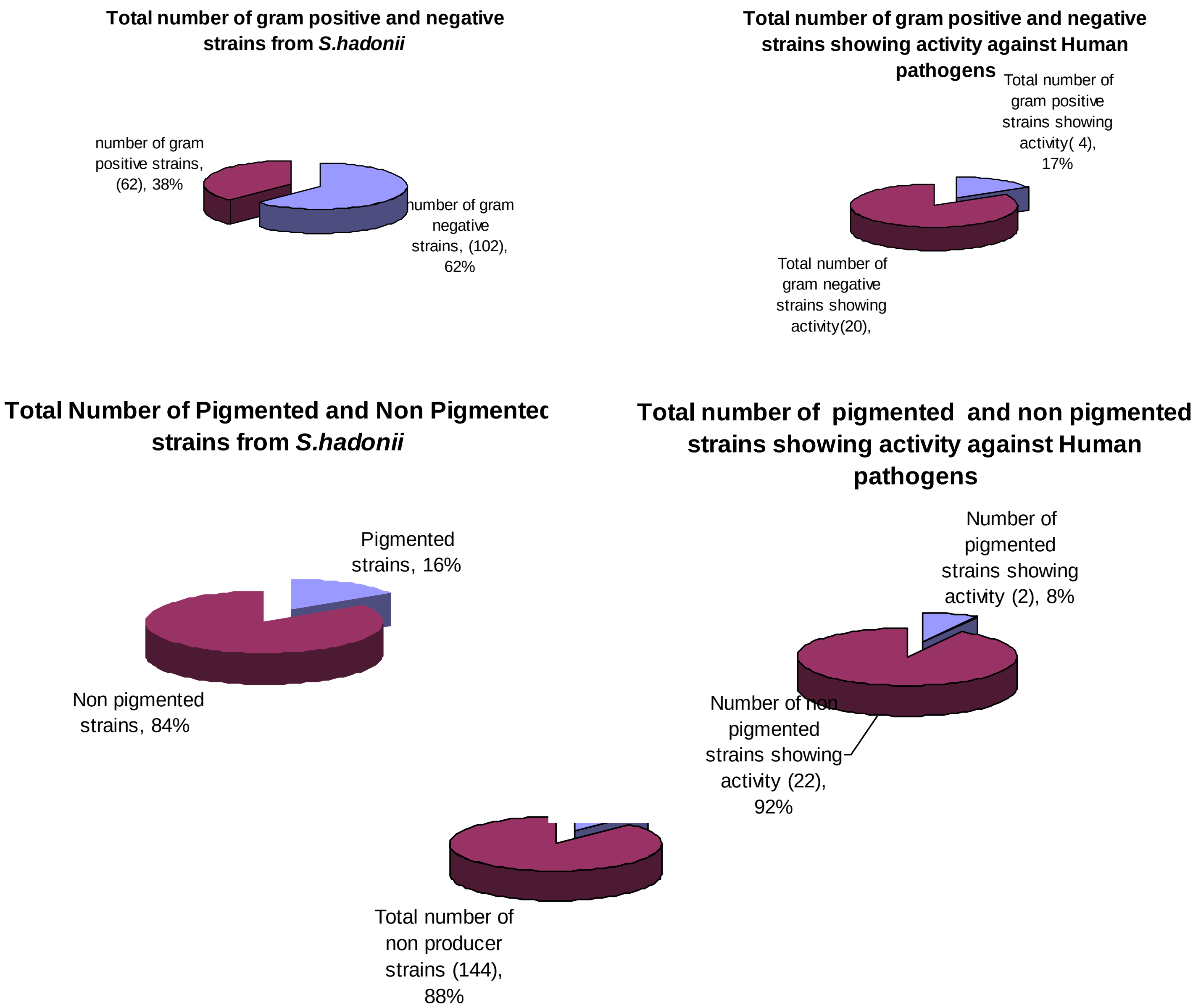
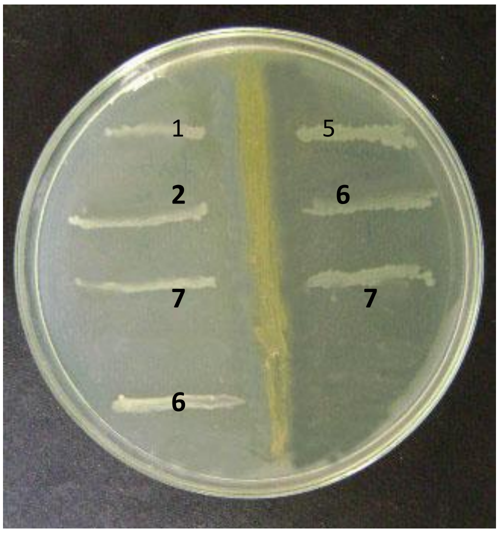
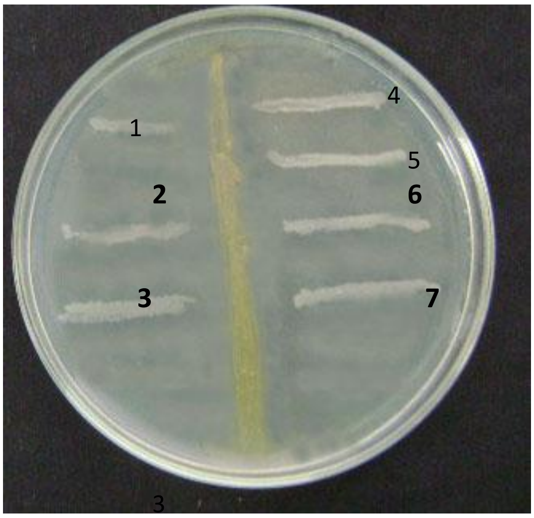


Fig. 6. The isolated microbes from *S.hadonii* showing activity against Human pathogens



SH 31



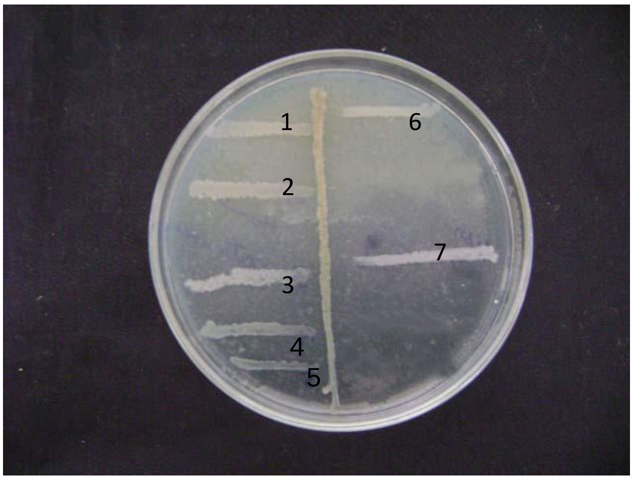
SH 60

SH 103

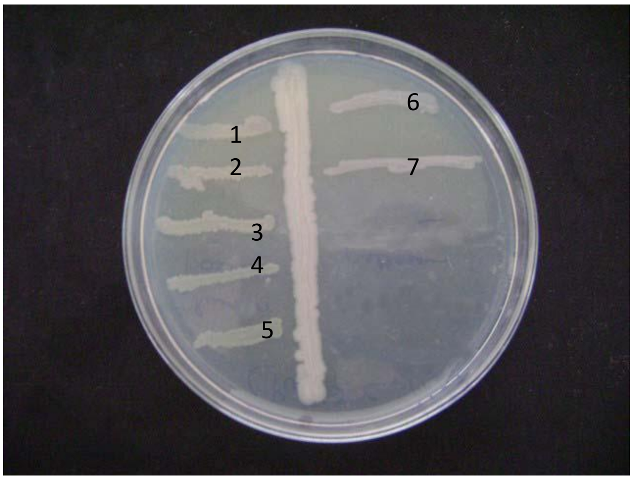
1. *E.coli;* 2.*V.harveyi;* 3.*E.aerugenosa;* 4.*P.pneumoniae;* 5.*V.cholerae;* 6.*S.typhi;* 7.*B.cereus*

Fig. 7. Antibacterial activity of sea anemone associated bacteria isolated from *Stichodactyla hadonii against* human pathogens

AE31



AE 63



AE 74

1. *V.harveyi*; 2.*A.hydrophyla* 3 .*P.mirabilis;* 4.*S.marcescens*; 5.*V.vulnificus*; 6.V.mimicus; 7.*Micrococcus* sp

Fig.8. Antibacterial activity of sea anemone associated bacteria isolated from *A. elegantissima* against fish pathogens

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