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Seahorses and pipefishes: looking into its antimicrobial potential as used in traditional chinese medicine (TCM)

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Abstract

Seahorses and pipefishes are important candidate organisms which have been used in Traditional Chinese Medicine (TCM). This study takes precedence in testing the antimicrobial potential of yellow seahorse (*Hippocampus kuda*) and scribbled pipefish (*Corythoichthys intestinalis*) crude extracts and decoction preparations with the following concentrations: 10%, 25%, 30%, 70% w/v. Kirby-Bauer disk diffusion test was used for the following test organisms: *Escherichia coli*, *Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis*. Observations from 24 to 72 hrs. were recorded. Photographs were taken for all the plates examined, processed and analyzed using UTHSCA Image tool software for measuring zones of inhibitions. Male and female pipefish crude extract preparations have effect on the test strains however, it yielded negative results on the decoction preparation. Inhibition zones were produced in all three (3) bacterial strains for the female pipefish while the male pipefish only produced inhibition zones against two (2) bacterial strains (*P. aeruginosa* and *S. aureus*). Noteworthy, various crude extract concentrations of male seahorse on *B. subtilis* and *E. coli* strikingly produced zones of inhibition. Varied concentrations of decoction preparation against *B. subtilis* and *S. aureus* also exhibit positive zones of inhibition. Noticeable zones of inhibition were commonly produced at 30 to 70% concentration for crude extracts. Results may support therapeutic claims albeit not comparable to commercial antibiotics. Moreover, it was observed that pipefishes classified as Least concern (LC) by IUCN produced more zones of inhibition thus, may alleviate pressure on rarer seahorses

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Introduction

Many marine organisms such as fishes have managed to survive a milieu of pathogenic organisms. For such, fishes had been utilized for the discovery and development of allopathic and medical treatment. It is believed that they yield novel compounds with unique chemical structures and significant biological activities. The chemical diversity of isolated compounds reflects the biodiversity of the source organisms which have evolved and adapted to their environment. Along this line, seahorses and pipefishes are important candidate organisms which have been used in Traditional Chinese Medicine since time immemorial.

In the marine environment, *Hippocampus* spp. (seahorses), are often found on varied habitats which include seagrass beds, mangroves, coral reefs and estuaries thus, considered as flagship species of various ecosystems (Vincent, 1996). So far there are 10 species of seahorses in the Philippines. Scientific researches revealed that these organisms exhibit biomedical properties like anti-aging, anti-fatigue, anti-cancer, anti-oxidant and antimicrobial. For this, dried seahorses had been exploited for trading and aquarium around 33 countries worldwide. Specific records with regard to medical uses of seahorses made populations vulnerable. Along this line, *Hippocampus kuda* is among the highly valued seahorse species in the Philippines, due to its smooth appearance and usually exhibiting pale yellow color that are much preferred by overseas markets of both traditional medicine and curio trade (Celino *et al.*, 2012). The International Union for the Conservation of Nature (IUCN) included *H. kuda* in its list of vulnerable species. To present, the supply of seahorses cannot meet the increasing market demand, especially with the increasing affluence of Asian consumers, the world's biggest market for seahorses (Vincent, 1996; Lourie *et al.*, 2004), thus placing sustainability and conservation matters at hand. Meanwhile, pipefishes are elongated fishes having a long and thin snout like a pipe with a body that seems to have a type of armor plating around it. They are allied to seahorses and there is still a dearth of studies pertaining to them. Many species are classified by IUCN as least concern (LC). *Corythoichthys intestinalis* commonly found on shallow sandy or mixed sand rubble or coral reefs and lagoons is among those classified as LC (Dawson, 1985) and abundant in the Philippines. A recent study also suggests pipefishes as a potential source of natural bioactive compounds (Priya *et al.*, 2013). If proven that common species of pipefishes that are least concern (LC) have potential antimicrobial activity they can help alleviate pressure on rarer seahorses. Hence, have implication on conservation.

In this respect, crude extracts and decoction preparation of seahorses and pipefishes gained popularity in Traditional Chinese Medicine (TCM) because of the quest of inexpensive alternate medicines compared to commercial drugs. Dried whole specimens were allowed to boil in water or pulverized and taken in the form of tea. However, the antimicrobial potential of the decoction preparation and crude extracts, respective dosage and concentrations remains ambiguous especially against normal microflora and potentially pathogenic bacterial strains, despite traditional use.

This study shed light on the antimicrobial potential of decoction preparations and crude extracts of varying concentrations of seahorse and pipefish species as used in Traditional Chinese Medicine (TCM). This study provided baseline data for future researches.

Materials and methods

Sample specimens

Seahorse and pipefish specimens were readily available in the laboratory at Premier Research Institute of Science and Technology (PRISM) and donated as dead bycatch samples from fishermen. Hence, such opportunity was taken advantage to study the antibacterial potential of seahorses and pipefishes. The seahorses were reported to come from Tubod, Lanao del Norte, Philippines and pipefishes were from Dalipuga, Iligan City. Identification of samples was done through illustrated keys, Guide to the identification of Seahorses (Lourie *et al.*, 2004) and consultation of experts. Maximum of two (2) individuals per species, per sex were used for the study since preparing the varying concentrations for the assay require minimal samples only. The study was conducted at the Premier Research Institute of Science and Technology (PRISM).

Test organisms

Strains of *Escherichia coli* BIOTECH 1634, *Pseudomonas aeruginosa* BIOTECH 1335, *Staphylococcus aureus* BIOTECH 1582, *Bacillus subtilis* BIOTECH 1679, were obtained from the University of the Philippines-Los Baños Biotechnology Laboratory.

These strains are potentially pathogenic and known to cause several human infections (Cheesbrough, 1984; Sabuda *et al*., 2008). These were subcultured on appropriate media as prescribed by BIOTECH, and incubated overnight at room temperature. A bacterial suspension for each test microorganisms was conducted using 0.5 McFarland Turbidity Standard, roughly $1.5x10^8$ cells/ml. These test organisms were used for preliminary screening of antimicrobial activity of associated organisms.

Crude extraction preparation

Fresh small pieces of tissue from seahorses and pipefishes specimen were crushed using mortar and pestle to obtain pure crude extract. The following concentrations 10%, 25%, 30%, 70% w/v were prepared by adding desired volume of sterilized distilled water and desired amount of crude extract. Volumes of respective prepared concentrations were used for the disk diffusion method.

Decoction preparation

Fresh small pieces of tissue from seahorses and pipefishes specimen were macerated using mortar and pestle. The following concentrations 10%, 25%, 30%, 70% w/v were prepared by adding desired volume of sterilized distilled water and allowed to boil for 30sec. to 1 min. Just like preparing tea as practiced in Traditional Chinese Medicine (TCM). Volumes of respective prepared concentrations were used for the disk diffusion method.

Kirby-Bauer disk diffusion test

For the Kirby-Bauer disk diffusion test protocol, bacterial suspension of the test microorganisms was set to 0.5 McFarland standard. Mueller-Hinton agar plates were seeded with a lawn of the following test organisms: *Escherichia coli* BIOTECH 1634, *Pseudomonas aeruginosa* BIOTECH 1335, *Staphylococcus aureus* BIOTECH 1582, and *Bacillus subtilis* BIOTECH 1679 for the antibacterial screening using sterile cotton swabs and employing aseptic techniques. Antibiotic disk tetracycline (30 µg) was used as the positive control while sterilized distilled water served as the negative control. After which these were dispensed on the agar surface along with the prepared seahorse and pipefish crude extract and decoction concentrations of 10%, 25%, 30%, 70% w/v. A volume of 30µl of each concentration was dispensed in sterilized blank discs of around 6mm in diameter and placed in the agar surface aseptically. The assay was performed in triplicates.

The resulting plates was incubated at 30°C for 24hrs and maximum of 72 hrs. Observations from 24 to 72 hrs. were recorded. Photographs were taken for all the plates examined with the ruler that served as a scale. Photographs were also processed and analyzed using UTHSCA Image tool software to measure the diameter of each zone of inhibition in millimeters.

Mean diameter in mm and standard deviation were generated and recorded by the software. Each zone measured in millimeters were evaluated for resistance or susceptibility using the comparative standard method. Antibiotic susceptibility was compared between the strains of microorganisms used (Bauer *et al*., 1966; Hudzicki, 2009).

Statistical analysis

Paleontological Statistics (PAST) v.2.17 software was used to analyze data. Statistical differences between the concentrations used were analyzed using one-way ANOVA and post hoc test, the Tukey's test, was done to check which groups were highly significant. P values lower than 0.05 ($p<0.05$) were considered significant.

Results and discussion

Fish have evolved a number of innate immune responses as defense against any type of infection. The defense may include elements such as antimicrobial peptides (Cole *et al.,* 1997) and antimicrobial lipid (Ravichandran *et al.,* 2009). Seahorses and pipefishes were known to possessed biomedical properties like anti-aging, anti-fatigue, anti-cancer, anti-oxidant and antimicrobial and for such reasons were highly exploited (Kumaravel *et al.*, 2010; Shapawi *et al.*, 2013; Sanaye *et al.*, 2014).

Table 1. Analysis of variance (ANOVA) for the zones of inhibition exhibited by 10% and 25% female pipefish crude extract concentrations and Tetracycline on *Bacillus subtilis*.

	Sum of sqrs	Df	Mean square	F	P
Between groups	122.204	2	61.1019	41.08	0.0003152
Within groups	8.92407	6	1.48734		
Total:	131.128	8			

Table 2. Analysis of variance (ANOVA) for the zones of inhibition exhibited by 30% and 70% female pipefish crude extract concentrations and Tetracycline on *Bacillus subtilis*.

	Sum of sqrs	Df	Mean square	F	P
Between groups	386.786	2	193.393	51.81	**0.000164**
Within groups	22.3961	6	3.73269		
Total:	409.182	8			

Table 3. Tukey's pairwise comparisons among 10% and 25% female pipefish crude extract on *Bacillus subtilis* that yield zones of inhibition versus the positive control Tetracycline.

	10%	25%	TETRACYCLINE
10%	-		
25%	0.9989	-	
TETRACYCLINE	0.0007411	0.0007238	-

Table 4. Tukey's pairwise comparisons among 30% and 70% female pipefish crude extract on *Bacillus subtilis* that yield zones of inhibition versus the positive control Tetracycline.

	30%	70%	TETRACYCLINE
30%	-		
70%	0.9739	-	
TETRACYCLINE	0.0004995	0.0004623	-

Results show an increasing trend of the concentrations of the male and female pipefish crude extract preparation that yield an effect on the four (4) test strains: *Bacillus subtilis, Pseaudomonas aeruginosa, Staphylococcus aureus and Escherichia coli* however, it yielded negative results on the decoction preparation. It was observed that the sex of the pipefish has influence on its antibacterial potential.

Inhibition zones were produced in three (3) bacterial strains in the test for antibacterial property of the female pipefish (Fig. 1) while the male pipefish only produced inhibition zones against two (2) bacterial strains *P. aeruginosa* and *S. aureus* (Fig. 2). Zones of inhibition were observed albeit not comparable to commercial antibiotic. Herewith, this shows the potential of varying concentrations of crude extracts of pipefishes.

Table 5. Analysis of variance (ANOVA) for the zones of inhibition exhibited by 10% and 25% female pipefish crude extract concentrations and Tetracycline on *Pseudomonas aeruginosa*.

	Sum of sqrs	Df	Mean square	F	P
Between groups	240.232	2	120.116	1156	1.732E-08
Within groups	0.6232	6	0.103867		
Total:	240.855	8			

Table 6. Analysis of variance (ANOVA) for the zones of inhibition exhibited by 30% and 70% female pipefish crude extract concentrations and Tetracycline on *Pseudomonas aeruginosa*.

	Sum of sqrs	Df	Mean square	F	P
Between groups	230.214	2	115.107	36.78	**0.0004291**
Within groups	18.7802	6	3.13003		
Total:	248.994	8			

Table 7. Tukey's pairwise comparisons among 10% and 25% female pipefish crude extract on *Pseudomonas aeruginosa* that yield zones of inhibition versus the positive control Tetracycline.

	10%	25%	TETRACYCLINE
10%	-		
25%	0.5269	-	
TETRACYCLINE	0.0002629	0.0002629	-

Table 8. Tukey's pairwise comparisons among 30% and 70% female pipefish crude extract on *Pseudomonas aeruginosa* that yield zones of inhibition versus the positive control Tetracycline.

	30%	70%	TETRACYCLINE
30%	-		
70%	0.9842	-	
TETRACYCLINE	0.000974	0.000885	-

Optimal activity was in 30% and 70% concentration. Results coincide with a study by Priya *et al.*, 2013, antimicrobial activity of pipefishes in methanol extract was tested against important bacterial and fungal pathogenic strains. In addition, studies reveal results of FT-IR showing that pipefish extracts of *Centriscus scutatus* and *Hippichthys cyanospilos* comprised of peptide derivatives as their predominant chemical groups suggesting pipefishes also as a potential source of natural bioactive compounds.

Table 9. Analysis of variance (ANOVA) for the zones of inhibition exhibited by 30% and 70% male pipefish crude extract concentrations and Tetracycline on *Staphylococcus aureus*.

	Sum of sqrs	Df	Mean square	F	P
Between groups	589.998	2	294.999	10.69	**0.01053**
Within groups	165.851	6	27.6084		
Total:	755.649	8			

Table 10. Analysis of variance (ANOVA) for the zones of inhibition exhibited by 25% male pipefish crude extract concentration and Tetracycline on *Pseudomonas aeruginosa*.

	Sum of sqrs	Df	Mean square	F	P
Between groups	620.37	1	620.37	36.18	**0.003846**
Within groups	68.5783	4	17.1446		
Total:	688.948	5			

Moreover, using varied concentrations of crude extract and decoction preparation, the antibacterial activity of the male and female seahorses *(Hippocampus kuda)* were tested. After 72 hours of incubation, only the male seahorse yields positive results against three (3) test strains. Zones of inhibition were observed albeit not comparable to commercial antibiotic. Increasing the concentration of the crude extract and decoction preparation of female seahorse do not have any effect on the test strains. Male seahorse strikingly produced mean zones of inhibition. Formation of inhibition zones were produced by various crude extract concentrations on *B. subtilis* and *E. coli* (Fig. 3).

Table 12. Tukey's pairwise comparison 25% male pipefish crude extract on *Pseudomonas aeruginosa* that yield zone of inhibition versus the positive control Tetracycline.

	25%	TETRACYCLINE
25%	-	
TETRACYCLINE	0.00403	-

Table 13. Analysis of variance (ANOVA) for the zones of inhibition exhibited by 10% and 25% male seahorse crude extract concentrations and Tetracycline on *Bacillus subtilis*.

	Sum of sqrs	Df	Mean square	F	P
Between groups	853.566	2	426.783	45.01	0.0002441
Within groups	56.8971	6	9.48286		
Total:	910.463	8			

Table 14. Analysis of variance (ANOVA) for the zones of inhibition exhibited by 10% and 25% male seahorse crude extract concentrations and Tetracycline on *Escherichia coli*.

	Sum of sqrs	Df	Mean square	F	P
Between groups	1183.24	2	591.619	147.8	7.871E-06
Within groups	24.015	6	4.0025		
Total:	1207.25	8			

Table 15. Analysis of variance (ANOVA) for the zones of inhibition exhibited by 30% and 70% male seahorse crude extract concentrations and Tetracycline on *Escherichia coli*.

	Sum of sqrs	Df	Mean square	F	P
Between groups	898.425	2	449.212	2574	1.578E-09
Within groups	1.04727	6	0.174544		
Total:	899.472	8			

Concentrations of decoction preparation against *B. subtilis* and *S. aureus* also exhibit positive zones of inhibition (Fig. 4). The results imply that various concentrations of seahorse pure crude extract and decoction preparation maybe sufficient to release active compounds and substances present in the male seahorse (*H. kuda)* that are responsible for its antibacterial potential.

Table 16. Tukey's pairwise comparisons among 10% and 25% male seahorse crude extract on *Bacillus subtilis* that yield zones of inhibition versus the positive control Tetracycline.

	10%	25%	TETRACYCLINE
10%	-		
25%	0.9797	-	
TETRACYCLINE	**0.0002294**	**0.0002298**	-

Table 17. Tukey's pairwise comparisons among 10% and 25% male seahorse crude extract on *Escherichia coli* that yield zones of inhibition versus the positive control Tetracycline.

	10%	25%	TETRACYCLINE
10%	-		
25%	0.9518	-	
TETRACYCLINE	**0.0006809**	**0.000572**	-

The Antimicrobial property of the seahorse extracts reveals that they are high enough to bring the effect against bacterial pathogens. It may due to the incidence of bacterial presence in their habitat induce the seahorse to produce the antimicrobial compounds (Kumaravel*et al.,* 2010).

Table 18. Tukey's pairwise comparisons among 30% and 70% male seahorse crude extract on *Escherichia coli* that yield zones of inhibition versus the positive control Tetracycline.

	30%	70%	TETRACYCLINE
30%	-		
70%	0.2639	-	
TETRACYCLINE	0.0002269	0.0002269	-

Table 19. Analysis of variance (ANOVA) for the zones of inhibition exhibited by 10% and 25% male seahorse decoction and Tetracycline on *Bacillus subtilis* strain.

	Sum of sqrs	Df	Mean square	F	P
Between groups	1307.68	2	653.839	15.36	0.004363
Within groups	255.423	6	42.5706		
Total:	1563.1	8			

Table 20. Tukey's pairwise comparisons among 10% and 25% male seahorse decoction on *Bacillus subtilis* that yield zones of inhibition versus the positive control Tetracycline.

	10%	25%	TETRACYCLINE
10%	-		
25%	0.9975	-	
TETRACYCLINE	0.007567	0.007077	-

Table 21. Analysis of variance (ANOVA) for the zones of inhibition exhibited by 30% male seahorse decoction and Tetracycline on *Bacillus subtilis* strain.

	Sum of sqrs	Df	Mean square	F	P
Between groups	369.892	1	369.892	1.71	**0.2552**
Within groups	840.259	4	210.065		
Total:	1210.15	5			

Results may support therapeutic claims albeit not comparable to commercial antibiotics. Notably, the diversity of toxins, biological compounds and associated microorganisms can be affected by the organism's ecology (Kohn, 1959; Kohn,1968; Duda *et al.*, 2001; Remigio and Duda, 2008).

Table 22. Tukey's pairwise comparisons of 30% male seahorse decoction on *Bacillus subtilis* that yield zones of inhibition versus the positive control Tetracycline.

	30%	TETRACYCLINE
30%	-	
TETRACYCLINE	0.2554	-

Table 23. Analysis of variance (ANOVA) for the zones of inhibition exhibited by 10% and 25% male seahorse decoction and Tetracycline on *S. aureus.*

	10%	25%	TETRACYCLINE
10%	-		
25%	0.9791	-	
TETRACYCLINE	0.0002798	0.0002879	-

Thus, microhabitats of organisms are revered as factors also governing their biomedical potential. In addition, it was observed that pipefishes (*C. intestinalis*) classified as Least concern (LC) by the International Union for Conservation of Nature (IUCN) produced more zones of inhibition. Noteworthy, is that the antibacterial activity of pipefish crude extracts was more active compared to the seahorses based on the diameter of the explicit zones of inhibition.

Table 24. Tukey's pairwise comparisons among 10% and 25% male seahorse decoction on *S. aureus* that yield zones of inhibition versus the positive control Tetracycline.

	Sum of sqrs	Df	Mean square	F	P
Between groups	2662.25	2	1331.12	77.09	5.255E-05
Within groups	103.599	6	17.2665		
Total:	2765.85	8			

Table 25. Analysis of variance (ANOVA) for the zones of inhibition exhibited by 30% and 70% male seahorse decoction and Tetracycline on *S. aureus*.

	Sum of sqrs	Df	Mean square	F	P
Between groups	2382.69	2	1191.35	73.95	5.926E-05
Within groups	96.6605	6	16.2665		
Total:	2479.35	8			

Table 26. Tukey's pairwise comparisons among 30% and 70% male seahorse decoction on *S. aureus* that yield zones of inhibition versus the positive control Tetracycline.

	10%	25%	TETRACYCLINE
10%	-		
25%	0.966	-	
TETRACYCLINE	0.0002999	0.0003117	-

These findings post as good news for conservation since, it can alleviate pressure on rarer seahorses.

One-way analysis of variance (ANOVA) and Tukey's test

Results showed that there is a statistically significant difference between group means that resulted positive for producing inhibition zones as determined by One-way ANOVA. Table 1 and 2 showed that 10%, 25% (F= 41.08; p= 0.0003152) and 30%, 70% (F= 51.81; p= 0.000164) concentrations of female pipefish crude extract against *B. subtilis* although not comparable to Tetracycline. Different concentrations with respect to positive control were further determined and confirmed which concentration differs using Tukey's pairwise comparison with p<0.05 as significant shown in Table 3 and 4. Significant difference between concentrations and positive control mean that there is an activity observed but not comparable to broad spectrum commercial antibiotic, the positive control.

Also, against *P. aeruginosa* varied concentrations crude extracts of female pipefish 10%, 25% (F= 1156 ; p=1.732E-08); 30%, 70% (F=36.78; p=0.0004291) have positive results but still not comparable to the positive control, Tetracycline as shown in Table 5 and 6. Concentrations with respect to positive control were further determined and confirmed which concentration differs using Tukey's pairwise comparison with p<0.05 as significant shown in Table 7 and 8.

The male pipefish only produced inhibition zones against two (2) bacterial strains *P. aeruginosa* and *S. aureus*. Against *S. aureus* varied concentrations of male pipefish crude extract: 30%, 70% (F= 10.69 and p=0.01053) shown in Table 9; against *Pseudomonas aeruginosa* at 25% concentration of male pipefish crude extract (F= 36.18 and p=0.003846) shown in

Table 10 have positive results but not comparable to the positive control Tetracycline. To further test which concentrations significantly differ Tukey's pairwise comparison with p<0.05 as significant shown in Tables 11 and 12 was done.

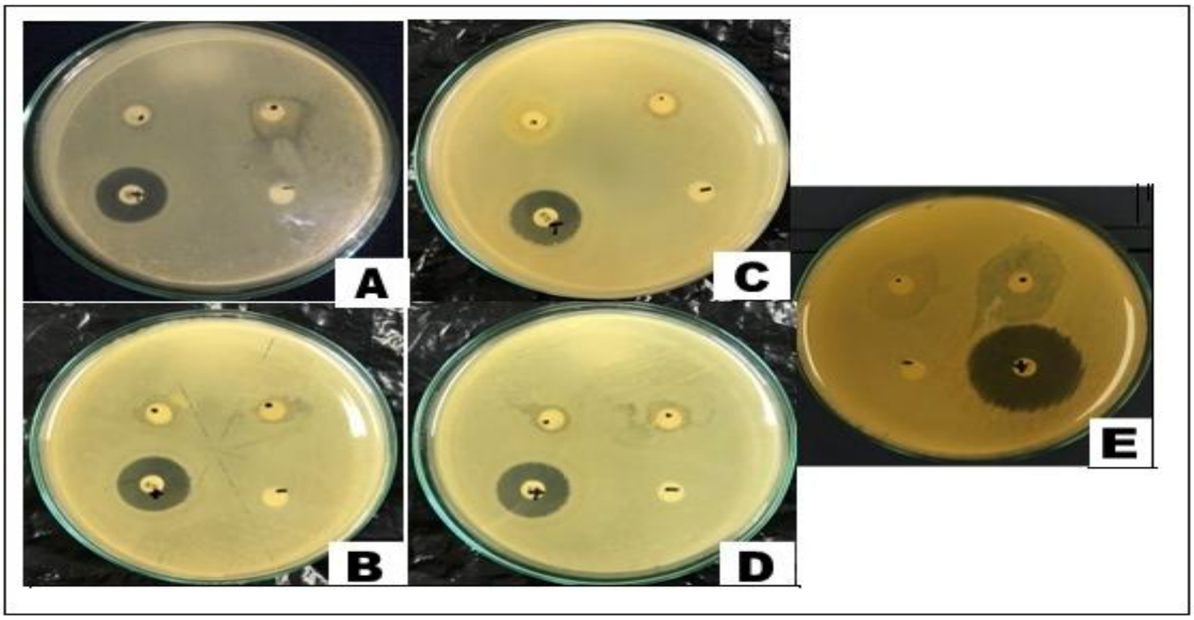


Fig. 1. Test for antibacterial activity of different concentrations of pure crude extract preparation of female pipefishes (*C. intestinalis*) using disc diffusion method. Varied concentrations of the crude extraction:(A) 10%(left), 25% (right) tested against *B. subtilis*; (B) 30% (left), 70% (right) tested against *B. subtilis;* (C) 10%(left),25% (right) against *P. aeruginosa;* (D) 30% (left), 70% (right) tested against *P. aeruginosa*;Tetracycline as positive control (positioned on the lower left most part of the plate) and distilled water as negative control (positioned on the lower right most part of the plate); (E) 30% (left), 70% (right) tested against *E. coli* after 72 hours of incubation. Tetracycline as positive control (positioned on the lower right most part of the plate) and distilled water as negative control (positioned on the lower left most part of the plate).

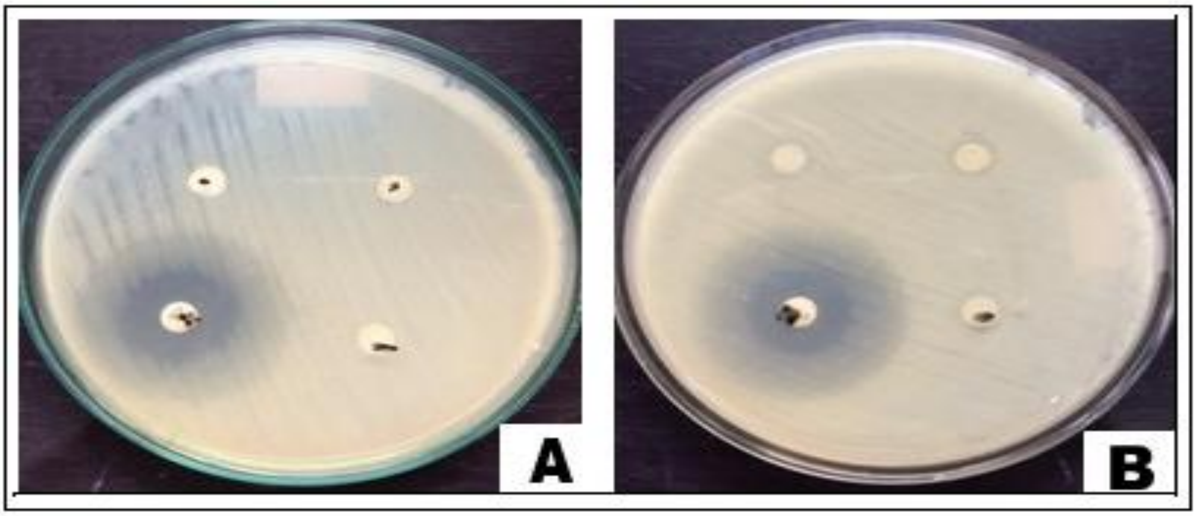


Fig. 2. Test for antibacterial activity of different concentrations of crude extract preparation of male pipefish using disc diffusion method after 72 hrs of incubation. Varied concentrations of the crude extraction: (A) 25% (right) tested against *Pseudomonas aeruginosa*, and (B) 30% (left),70% (right) tested against *Staphylococus aureus*. Tetracycline as positive control (positioned on the lower right most part of the plate) and distilled water as negative control (positioned on the lower right most part of the plate).

Male seahorse in crude extract preparation yielded positive results with 10% and 25% (F= 45.01 and p= 0.0002441) against *B. subtilis* and 10%, 25% (F=147.8, p= 7.871E-06) 30%, 70% (F= 2574, p= 1.578E-09) against *E. coli* (Tables 13 to 15). Tukey's pairwise comparison with p<0.05 as significant shown in Tables 16 to 18 was done to test which concentrations significantly differ to the positive control, Tetracycline.

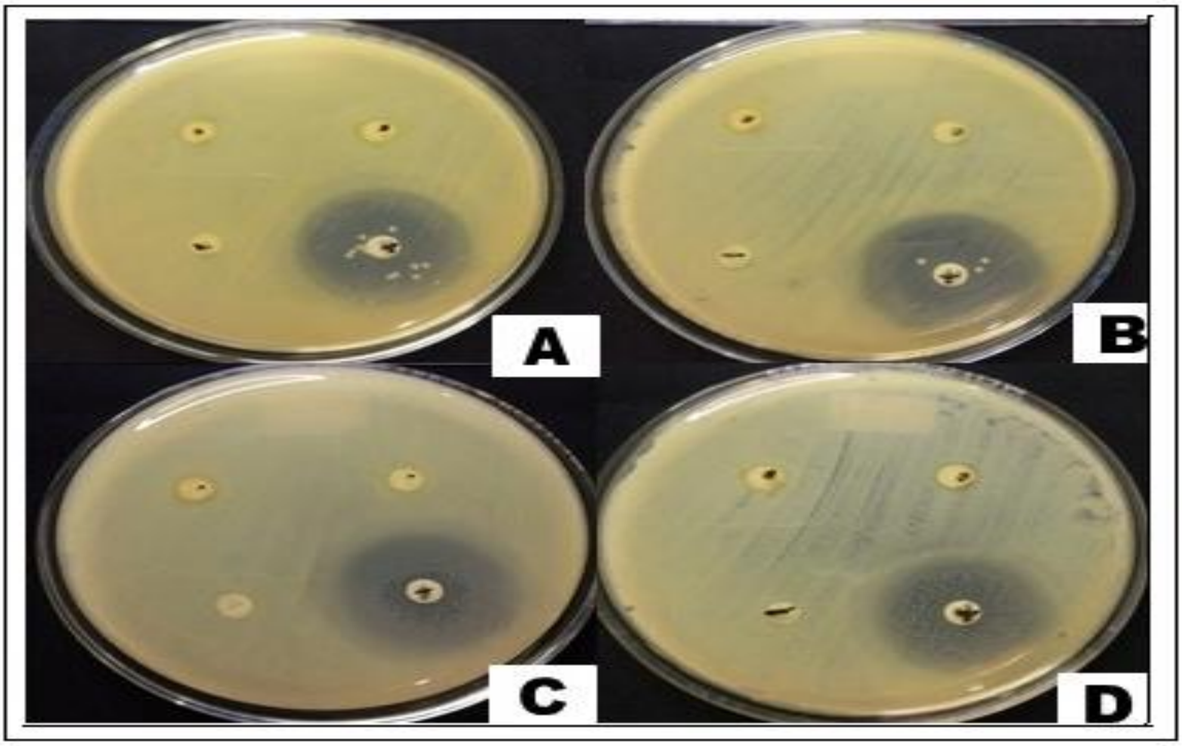


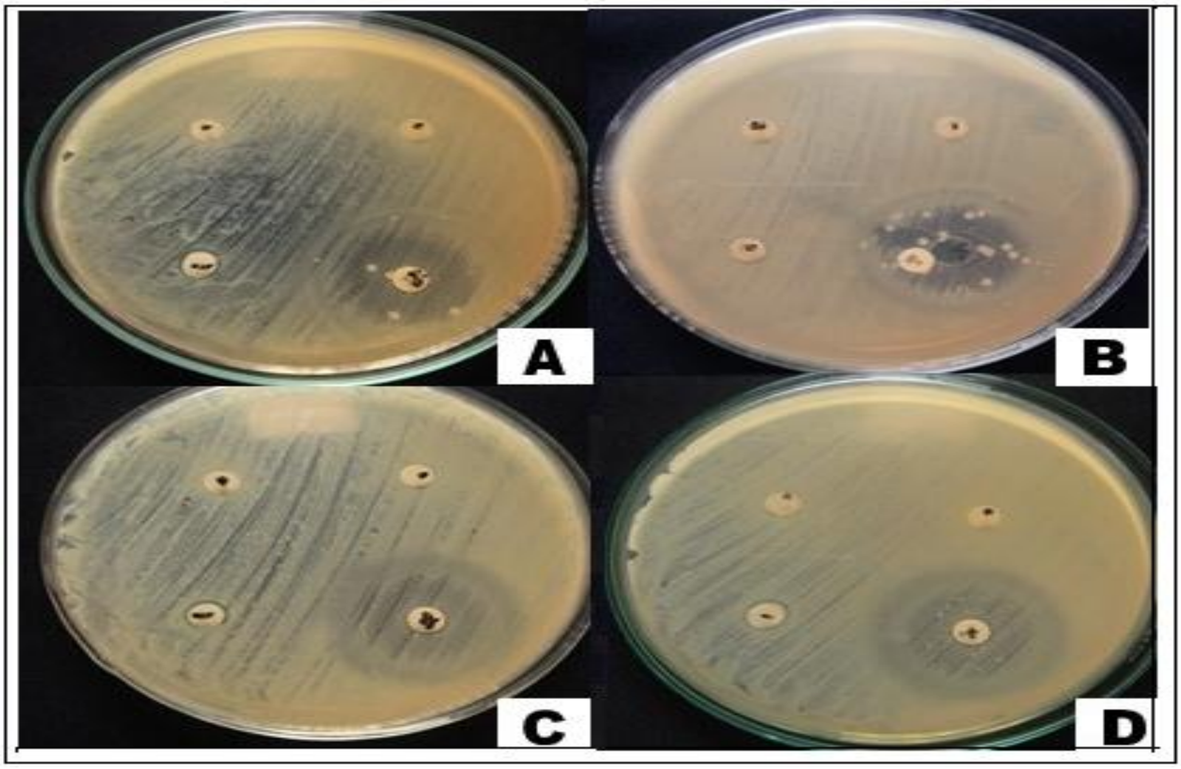
Fig. 3. Test for antibacterial activity of different concentrations of crude extract preparation of male seahorse using disc diffusion method. Varied concentrations of the crude extraction: (A) 10% (left), 25% (right) and (B) 30% (left), 70% (right) on *B. subtilis*, (C) 10% (left), 25% (right) and (D) 30% (left), 70% (right) on *E. coli* after 72 hours of incubation.Tetracycline as positive control (positioned on the lower right most part of the plate) and distilled water as negative control (positioned on the lower right most part of the plate).

Decoction preparation in male seahorse resulted positive in concentrations 10% and 25% (F= 15.36and p= 0.004363) and 30% with (F= 1.71 and p= 0.2552) against *Bacillus subtilis* strain and Tukey's pairwise comparison with p<0.05 as significant shown in Tables 19 to 22 was done to test which concentrations significantly differ to the positive control, Tetracycline. On the other hand, concentrations 10%, 25% ((F=77.09, p= 5.255E-05) and concentrations 30%, 70% (F=73.95, p= 5.926E-05) as shown in Tables 24 to 26 yielded positive results against *S. aureus* strain.

Thus, results indicate varied concentrations produced clearing zones albeit not comparable to commercialized broad spectrum antibiotic Tetracycline. The results obtained were still relevant since there are concentrations which displayed potential antibacterial activity.

In this connection, a study by Al-Rasheed, 2018 states that epidermal mucus of fish possess a number of biologically active constituents including antimicrobial peptides.

These are continuously expressed and involved in the provision of protection to the fish against injurious substances as well as potential pathogenic microbes. The innate immune response is essential for survival and defense includes elements such as antimicrobial peptides, antimicrobial lipid and polypeptides (Cole *et al.*, 1997; Ellis, 2001; Plouffe *et al.*, 2005; Magnadottir, 2006; Ravichandran *et al.*, 2010). Fishes signify an important source of functional materials like polyunsaturated fatty acids, polysaccharides, high quality peptides, proteins, lipids, antioxidants, enzymes and a wide variety of vitamins and minerals (Khora, 2013).



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ig. 4. Test for antibacterial activity of different concentrations of decoction preparation of male seahorse using disc diffusion method. Varied concentrations of the decoction preparation: (A) 10% (left),25% (right); (B)30% (left) tested against *B. subtilis*; (C,D) 10% (left), 25% (right); 30% (left), 70% (right)tested against *S. aureus*. Tetracycline as positive control (positioned on the lower right most part of the plate) and distilled water as negative control (positioned on the lower right most part of the plate).

In Brazil, 283 species of animals have been recorded to have medicinal values and 81 species of these are of fish-origin which includes seahorses and allies (Alves *et al.*, 2007). Herewith, crude extracts in its form might possess such substances as still active to elicit a response in the assay performed. Drying specimens and preparation of tea as practiced by Traditional Chinese Medicine (TCM) is not recommended if one is after unleashing the antimicrobial potential of seahorses and pipefishes. Decoction seems to reduce the effectivity. Since, crude extracts display a potential albeit not comparable to commercial antibiotic and results can be obtained in preparations with minimal samples needed and not at higher concentrations, it can be developed and recommended for external applications (like ointments). In this manner, conservation can also be done since minimal amount is only required.

Conclusion

This study takes precedence in testing the antimicrobial potential of crude extract and decoction preparation of varied concentrations of yellow seahorse (*Hippocampus kuda*) and scribbled pipefish (*Corythoichthys intestinalis*). Results indicate varied concentrations produced clearing zones albeit not comparable to commercialized broad spectrum antibiotic, Tetracycline.

The results obtained were still relevant since, there are concentrations which displayed potential antibacterial activity. Noticeable zones of inhibition were commonly produced at 30 to 70% concentration for crude extracts. Decoction seems to reduce the effectivity.

The scribbled pipefish (*Corythoichthys intestinalis*) crude extracts displayed more activity compared to seahorses which may have implications to conservation, it being classified as least concern (LC) by IUCN. Moreover, since crude extracts display a potential albeit not comparable to commercial antibiotic, it can be developed and recommended for external applications (like ointments).

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