



Evaluation of antioxidant and antimicrobial properties of the muscle extracts of *Chrysochir aureus* and *Otolithes ruber* from Kakinada Harbour, Andhra Pradesh, India

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ABSTRACT

Marine fish are important sources of bioactive compounds with potential pharmaceutical and nutraceutical applications. This study evaluated the antioxidant and antimicrobial activities of muscle extracts from *Chrysochir aureus* and *Otolithes ruber*. Muscle tissues were extracted using organic solvents and analyzed for antioxidant activity through standard in vitro assays, including free radical scavenging and reducing power tests. Antimicrobial activity was assessed against selected Gram-positive and Gram-negative bacteria, as well as fungal pathogens, using the agar well diffusion method. Both fish muscle extracts exhibited concentration-dependent antioxidant activity, with *Chrysochir aureus* demonstrating greater free radical scavenging potential than *Otolithes ruber*. The extracts also showed broad-spectrum antimicrobial activity, producing varying degrees of inhibition against the tested microorganisms. These bioactivities are likely associated with naturally occurring bioactive peptides, proteins, and other antioxidant constituents present in the muscle tissue. The findings indicate that muscle extracts of *Chrysochir aureus* and *Otolithes ruber* are promising natural sources of antioxidant and antimicrobial compounds with potential applications in food preservation, pharmaceuticals, and functional foods. Further studies focusing on the isolation, characterization, and in vivo evaluation of these bioactive compounds are needed to confirm their therapeutic potential and elucidate their mechanisms of action.

Keywords: Antioxidant activity, Antimicrobial activity, *Chrysochir aureus*, *Otolithes ruber*

INTRODUCTION

Antimicrobial resistance (AMR) and oxidative stress are major global health challenges that reduce the effectiveness of conventional antibiotics and contribute to chronic diseases such as cancer, diabetes, cardiovascular, and neurodegenerative disorders (World Health Organization, 2020; Lobo et al., 2010). The ability of biofilm-forming pathogens to resist antimicrobial agents further complicates treatment strategies (Nudelman et al., 2021), driving the need for novel natural bioactive compounds.

Marine ecosystems are a rich source of structurally diverse metabolites with pharmaceutical potential, including antimicrobial peptides, proteins, collagen, and antioxidant molecules that support immune defense and environmental adaptation (Costello et al., 2010; Jafari et al., 2020; El-Seedi et al., 2025). In this context, fish species such as *Chrysochir aureus* and *Otolithes ruber* remain underexplored despite their commercial importance.

Therefore, this study investigates the antioxidant and antimicrobial activities of muscle extracts from *C. aureus* and *O. ruber* to assess their potential as natural sources of bioactive compounds for pharmaceutical and nutraceutical applications.

Pathogenic bacterial contamination poses a significant global public health challenge, impacting agriculture, food production, healthcare, and environmental sustainability (Shi et al., 2023). Biofilm-associated infections account for nearly 80% of bacterial infections and exhibit 10–1000-fold greater resistance to antibiotics and disinfectants than planktonic cells, complicating treatment and control strategies (Nudelman et al., 2021). The escalating prevalence of antimicrobial resistance (AMR), driven largely by the overuse and misuse of antibiotics, further threatens the effective management of infectious diseases and contributes to increased morbidity, mortality, and healthcare costs (World Health Organization, 2020; Ventola, 2015). Concurrently, oxidative stress resulting from excessive production of reactive oxygen species (ROS) plays a central role in the pathogenesis of numerous chronic disorders, including cancer, cardiovascular diseases, diabetes, and neurodegenerative conditions, through oxidative damage to cellular biomolecules (Valko et al., 2007; Lobo et al., 2010). These challenges underscore the urgent need to identify novel natural compounds with both antimicrobial and antioxidant properties.

Marine ecosystems constitute a rich reservoir of structurally diverse bioactive molecules with considerable pharmaceutical potential. Owing to their unique physiological adaptations, marine organisms synthesize a wide range of secondary metabolites, including alkaloids, terpenoids, peptides, and proteins, exhibiting antimicrobial, antioxidant, anti-inflammatory, and anticancer activities (Costello et al., 2010; El-Seedi et al., 2025).

Among these, marine fish have emerged as promising sources of bioactive compounds such as collagen and antimicrobial peptides, particularly from skin and scales, with demonstrated applications in wound healing and tissue engineering (Jafari et al., 2020). Furthermore, exploring the bioactive potential of marine fish supports both the discovery of novel therapeutic agents and the sustainable utilization of marine biodiversity (Imen et al., 2017). Therefore, the present study investigated the antioxidant and antimicrobial activities of extracts obtained from the marine fish species *C. aureus* and *O. ruber*.

MATERIALS AND METHODS

Evaluation of Medicinal value:

The medicinal significance of selected fish species such as *C. aureus* and *O. ruber* were evaluated by the estimation of antioxidant and antimicrobial properties.

Solvent Extract Preparation:

Fresh muscle tissue (100 g) was dried at 50°C, powdered, and 10 g of each sample was macerated in chloroform: methanol (1:1, 200 ml) for 8 days at room temperature in dark conditions with intermittent stirring. The extract was filtered (Whatman No.1), concentrated using Soxhlet extraction and rotary evaporation, and then freeze-dried.

$$\% \text{ Extract yield} = \frac{m_2}{m_1} \times 100$$

m_1 = weight of dried muscle tissue,

m_2 = weight of freeze-dried extract

The dried extract was stored at 4°C in airtight amber bottles.

Preparation of Extract Concentrations:

A stock solution (100 mg/ml) was prepared by dissolving 1 g extract in 10 ml DMSO. From this, serial dilutions were prepared: 10, 5.0, 2.5, 1.25, and 0.625 mg/ml

Antioxidant Assays:

All assays were performed using increasing extract concentrations.

DPPH Radical Scavenging Activity (Mensor et al., 2001)

$$\% \text{ DPPH scavenging} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

Measured at 517 nm after 30 min incubation in dark.

ABTS Radical Scavenging Activity (Shirwaikar et al., 2006)

$$\% \text{ ABTS scavenging} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

Measured at 734 nm after 30 min incubation.

FRAP Assay (Benzie & Strain, 1996)

$$\% \text{ FRAP activity} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

Results expressed as FeSO₄ equivalents; absorbance read at 593 nm.

Superoxide Scavenging Activity (Winterbourn et al., 1975)

$$\% \text{ Superoxide scavenging} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

Measured at 560 nm after riboflavin-light exposure.

Nitric Oxide Scavenging Activity (Balakrishnan et al., 2009)

$$\% \text{ NO scavenging} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

Measured at 546 nm using Griess reagent.

Total Antioxidant Capacity (TAC) (Prieto et al., 1999)

Extracts were incubated with phosphomolybdenum reagent at 95°C for 90 min.

TAC = mg ascorbic acid equivalents (AAE) per g of extract

Antimicrobial Activity:

Agar Well Diffusion Method

Antimicrobial activity was tested against:

Gram-positive bacteria: *Bacillus spizizenii*, *Streptococcus mutans*

Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*

Fungi: *Aspergillus niger*, *Candida albicans*

Extract concentrations used: 10–0.625 mg/ml

Controls: erythromycin (bacteria), nystatin (fungi), DMSO (negative control)

Statistical Analysis:

All experiments were performed in triplicate. Data were expressed as mean ± standard deviation.

Statistical analysis was performed using one-way ANOVA and regression-correlation analysis in SPSS 10.0. Significance level: $p < 0.05$.

RESULTS

Percentage Yield of Extracts:

The percentage yield of chloroform: methanol muscle extracts was 15.7% for *C. aureus* and 13.4% for *O. ruber*. The relatively higher yield obtained using a polar-non-polar solvent system indicates efficient extraction of diverse bioactive compounds, including both polar and non-polar constituents. Variations in yield may depend on solvent type, extraction time, temperature, and solvent-to-sample ratio, as previously reported (Quispe-Condori et al., 2008; Terblanche et al., 2017).

Antioxidant Potential:

Antioxidant activity of both fish extracts was evaluated using DPPH, ABTS, FRAP, superoxide, and nitric oxide scavenging assays at concentrations ranging from 0.625–10 mg/ml. All results are presented as mean ± SD (n=3), and analyzed using one-way ANOVA ($p < 0.05$).

DPPH Radical Scavenging Activity:

DPPH scavenging activity increased significantly with increasing extract concentration in both species, indicating a dose-dependent antioxidant effect.

C. aureus: 15.15 ± 1.29% to 76.67 ± 2.22%

O. ruber: 8.46 ± 0.72% to 64.46 ± 0.89%

The IC₅₀ values were 4.46 mg/ml (*C. aureus*) and 6.47 mg/ml (*O. ruber*), indicating stronger activity in *C. aureus*.

The results show that *C. aureus* exhibited higher radical scavenging potential across all concentrations. The activity was statistically significant ($p < 0.05$) and showed strong positive correlation with concentration ($r=0.918$ for *C. aureus*, $r=0.923$ for *O. ruber*), confirming a clear dose-dependent antioxidant response.

ABTS radical scavenging activity:

ABTS assay was used to evaluate the antioxidant potential of muscle extracts from *C. aureus* and *O. ruber*. The results showed a significant, concentration-dependent increase in ABTS radical scavenging activity for both species, indicating stronger antioxidant performance at higher extract concentrations.

C. aureus exhibited higher ABTS scavenging activity at all tested concentrations (0.625–10 mg/ml), ranging from $42.08 \pm 1.62\%$ to $69.41 \pm 0.95\%$, whereas *O. ruber* showed values from $28.12 \pm 1.46\%$ to $62.30 \pm 1.06\%$. The IC_{50} values were 2.04 mg/ml for *C. aureus* and 5.49 mg/ml for *O. ruber*, indicating stronger activity in

C. aureus. The results were statistically significant ($P < 0.05$) and showed strong positive correlations with concentration ($r = 0.952$ for *C. aureus*, $r = 0.948$ for *O. ruber*), confirming a clear dose-dependent antioxidant response.

Table 1: Percentage of DPPH radical scavenging activities from five selected fish species with increasing concentrations

S. No.	Extract (mg/ml)	Conc.	% of DPPH inhibition (Mean $\pm$ SD)		t- value	df- value	p - value
			<i>Chrysochir aureus</i>	<i>Otolithes ruber</i>			
1	0.625		15.15 $\pm$ 1.29	8.46 $\pm$ 0.72	7.8435	4	0.0014
2	1.25		28.22 $\pm$ 1.84	15.74 $\pm$ 1.13	10.0107	4	0.0006
3	2.5		46.01 $\pm$ 1.89	38.93 $\pm$ 1.79	4.7109	4	0.0092
4	5		65.9 $\pm$ 1.72	49.84 $\pm$ 1.51	12.1535	4	0.0003
5	10		76.67 $\pm$ 2.22	64.46 $\pm$ 0.89	8.8422	4	0.0009

$P < 0.05$ was considered as significant difference.

Table 2: Correlation of DPPH radical scavenging activities from two selected fish species with increasing concentrations

Type of Extract	Extract conc.	<i>Chrysochir aureus</i>	<i>Otolithes ruber</i>
<i>Chrysochir aureus</i>	0.918089	1	
<i>Otolithes ruber</i>	0.923456	0.990733	1

Table 3: Percentage of ABTS radical scavenging activities from the selected fish species with increasing concentrations

S. No.	Extract Conc. (mg/ml)	% of ABTS inhibition (Mean $\pm$ SD)		t- value	df- value	p- value
		<i>Chrysochir aureus</i>	<i>Otolithes ruber</i>			
1	0.625	42.08 $\pm$ 1.62	28.12 $\pm$ 1.46	11.0873	4	0.0004
2	1.25	47.56 $\pm$ 0.75	36.36 $\pm$ 1.04	15.1292	4	0.0001
3	2.5	54.74 $\pm$ 1.29	44.31 $\pm$ 1.25	10.430	4	0.0005
4	5	61.11 $\pm$ 1.09	52.03 $\pm$ 1.27	9.080	4	0.0007
5	10	69.41 $\pm$ 0.95	62.3 $\pm$ 1.06	8.6517	4	0.001

$P < 0.05$ was considered as significant difference

Table 4: Correlation of ABTS radical scavenging activities from two selected fish species with increasing concentrations

Type of Extract	Extract conc.	<i>Chrysochir aureus</i>	<i>Otolithes ruber</i>
<i>Chrysochir aureus</i>	0.952984	1	
<i>Otolithes ruber</i>	0.948194	0.999171	1

FRAP assay

FRAP assay was used to evaluate the ferric ion reducing ability of muscle extracts from *C. aureus* and *O. ruber*. Results showed a significant, concentration-dependent increase in reducing power for both species.

C. aureus showed FRAP values ranging from $12.81 \pm 0.95\%$ to $72.89 \pm 1.46\%$, while *O. ruber* ranged from $19.24 \pm 0.82\%$ to $67.10 \pm 1.34\%$ at concentrations of

0.625–10 mg/ml. Both species exhibited comparable ferric reducing activity.

The EC_{50} values were 5.34 mg/ml (*C. aureus*) and 5.72 mg/ml (*O. ruber*), indicating slightly higher activity in *C. aureus*. The results were statistically significant ($p < 0.05$) and showed strong positive correlations with concentration ($r = 0.943$ for *C. aureus*, $r = 0.948$ for *O. ruber*), confirming dose-dependent antioxidant potential.

Table 5: Percentage of FRAP activities from the selected fish species with increasing concentrations

S. No.	Extract (mg/ml)	Conc.	% of FRAP activity (Mean±SD)		t-value	df- value	p- value
			<i>Chrysochir aureus</i>	<i>Otolithes ruber</i>			
1	0.625		12.81±0.95	19.24±0.82	8.8745	4	0.0009
2	1.25		21.89±1.09	22.3±1.48	0.3864	4	0.7189
3	2.5		42.2±1.31	37.89±0.98	4.5630	4	0.0103
4	5		55.85±1.04	55.57±1.09	0.3219	4	0.7636
5	10		72.89±1.46	67.1±1.34	5.0605	4	0.0072

P<0.05 was considered as significant difference

Table 6: Correlation of FRAP activities from two selected fish species with increasing concentrations

Type of Extract	Extract conc.	<i>Chrysochir aureus</i>	<i>Otolithes ruber</i>
<i>Chrysochir aureus</i>	0.943134	1	
<i>Otolithes ruber</i>	0.948582	0.991219	1

Table 7: Percentage of superoxide scavenging activities from selected fish species with increasing concentrations

S. No.	Extract (mg/ml)	Conc.	% of Superoxide inhibition (Mean ± SD)		t-value	df- value	p- value
			<i>Chrysochir aureus</i>	<i>Otolithes ruber</i>			
1	0.625		7.16±0.58	3.23±0.39	9.7391	4	0.0006
2	1.25		13.45±0.64	7.77±0.37	13.3080	4	0.0002
3	2.5		22.59±0.9	17.57±0.73	7.5031	4	0.0017
4	5		37.39±1.13	24.92±1.18	13.2199	4	0.0002
5	10		60.01±1.28	46.2±1.47	12.2716	4	0.0003

P <0.05 was considered as significant difference

Table 8: Correlation of superoxide scavenging activities from two selected fish species with increasing concentrations

Type of Extract	Extract conc.	<i>Chrysochir aureus</i>	<i>Otolithes ruber</i>
<i>Chrysochir aureus</i>	0.992581	1	
<i>Otolithes ruber</i>	0.991153	0.995243	1

Superoxide scavenging activity

Superoxide scavenging assay was used to evaluate the ability of muscle extracts from *C. aureus* and *O. ruber* to neutralize reactive oxygen species. The activity increased significantly in a dose-dependent manner for both species.

C. aureus showed higher activity, ranging from 7.15 ± 0.58% to 60.01 ± 1.28%, while *O. ruber* ranged from 3.23 ± 0.39% to 46.20 ± 1.47% at concentrations of 0.625–10 mg/ml.

The IC₅₀ values were 7.85 mg/ml (*C. aureus*) and 10.7 mg/ml (*O. ruber*), indicating stronger activity in *C. aureus*. The results were statistically significant ($p < 0.05$) and showed very strong positive correlations with concentration ($r = 0.992$ for *C. aureus*, $r = 0.991$

for *O. ruber*), confirming a clear dose-dependent antioxidant response.

Nitric oxide scavenging activity

Nitric oxide scavenging assay was used to evaluate the ability of muscle extracts from *C. aureus* and *O. ruber* to neutralize nitric oxide and other reactive nitrogen species. The activity increased significantly in a dose-dependent manner for both species.

O. ruber showed higher Nitric Oxide (NO) scavenging activity than *C. aureus* at all tested concentrations (0.625–10 mg/ml).

- *C. aureus* showed 38.96 ± 1.22% to 74.75 ± 0.98%
- *O. ruber* showed 42.63 ± 0.90% to 82.38 ± 1.07%

The results were statistically significant ($p < 0.05$), confirming a strong concentration-dependent increase in activity.

Table 9: Percentage of nitric oxide scavenging activities from selected fish species with increasing concentrations

S. No.	Extract (mg/ml)	Conc.	% of Nitric oxide inhibition (Mean±SD)		t-value	df- value	p- value
			<i>Chrysochir aureus</i>	<i>Otolithes ruber</i>			
1	0.625		38.96±1.22	42.63±0.9	5.8054	4	0.0044
2	1.25		45.4±0.69	56.58±0.65	20.4277	4	0.0001
3	2.5		55.34±0.88	65.77±1.07	13.0399	4	0.0002
4	5		62.28±1.52	71.11±1.42	7.3525	4	0.0018
5	10		74.75±0.98	82.38±1.07	9.1081	4	0.0008

$p < 0.05$ was considered as significant difference

Table 10: Correlation of nitric oxide scavenging activities from two selected fish species with increasing concentrations

Type of Extract	Extract conc.	<i>Chrysochir aureus</i>	<i>Otolithes ruber</i>
<i>Chrysochir aureus</i>	0.960202	1	
<i>Otolithes ruber</i>	0.903964	0.981454	1

Total antioxidant activity (TAC):

Table 11 presents Total antioxidant capacity (TAC) of muscle extracts from *C. aureus* and *O. ruber* was evaluated using the phosphomolybdenum assay and expressed as % ascorbic acid equivalents (AAE). Both species showed a clear, concentration-dependent increase in antioxidant activity.

C. aureus exhibited TAC values ranging from $49.58 \pm 1.25\%$ to $87.87 \pm 1.48\%$, while *O. ruber* showed higher values ranging from $62.32 \pm 1.09\%$ to $90.89 \pm 1.42\%$ at 0.625–10 mg/ml, indicating comparatively stronger overall antioxidant potential in *O. ruber*.

The EC₅₀ values were 2.57 mg/ml (*C. aureus*) and 4.17 mg/ml (*O. ruber*). Statistical analysis showed significant differences between species at 0.625 mg/ml ($p = 0.0002$) and 5 mg/ml ($p = 0.0395$), while no significant differences were observed at other concentrations ($p > 0.05$).

A strong positive correlation was observed between concentration and TAC for both species ($r = 0.831$ for *C. aureus*, $r = 0.955$ for *O. ruber*), confirming dose-dependent antioxidant behavior.

Table 11: Total antioxidant activity from the muscle extracts of the selected species with increasing concentrations

S. No.	Extract (mg/ml)	Conc.	% of AAE (Ascorbic acid equivalents) (Mean ± SD)		t-value	df- value	p- value
			<i>Chrysochir aureus</i>	<i>Otolithes ruber</i>			
1	0.625		49.58±1.25	62.32±1.09	13.3050	4	0.0002
2	1.25		64.22±1.31	67.88±1.91	2.7371	4	0.0521
3	2.5		74.78±1.27	74.38±0.88	0.4484	4	0.6771
4	5		86.38±1.0	83.07±1.62	3.0114	4	0.0395
5	10		87.87±1.48	90.89±1.42	2.5503	4	0.0633

$P < 0.05$ was considered as significant difference

Table 12: Correlation of Total antioxidant activity from two selected fish species with increasing concentrations

Type of Extract	Extract conc.	<i>Chrysochir aureus</i>	<i>Otolithes ruber</i>
<i>Chrysochir aureus</i>	0.831269	1	
<i>Otolithes ruber</i>	0.955104	0.957612	1

Antimicrobial activity:

The antimicrobial activity of muscle extracts from *Chrysochir aureus* and *Otolithes ruber* was evaluated using the well diffusion method at five concentrations (0.625, 1.25, 2.5, 5, and 10 mg/ml). Antibacterial activity was tested against Gram-positive bacteria (*Bacillus spizizenii*, *Streptococcus mutans*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), while antifungal activity was assessed against *Aspergillus niger* and *Candida albicans*.

Antimicrobial activity of *Chrysochir aureus*:

Muscle extracts of *Chrysochir aureus* exhibited maximum antimicrobial activity at 10 mg/ml against all tested microorganisms. The highest inhibition was observed against *P. aeruginosa* (34 mm), followed by *B. spizizenii* (33 mm), *E. coli* (33 mm), *S. mutans* (32 mm), *A. niger* (15 mm), and *C. albicans* (12 mm). At the lowest concentration (0.625 mg/ml), notable antibacterial activity was still observed against *P. aeruginosa* (27 mm), *E. coli* (22 mm), *B. spizizenii* (21 mm), and *S. mutans* (16 mm). However, antifungal activity against *A. niger* and *C. albicans* was detected only at 5 and 10 mg/ml, while lower concentrations (0.625–2.5 mg/ml) showed no antifungal effect. Table 13 and Figure 1 summarize the concentration-dependent antimicrobial activity of *C. aureus* extracts.

Correlation analysis (Table 14) indicated a strong positive relationship between extract concentration and antimicrobial activity across most organisms, including *S. mutans* ($r = 0.94949$), *E. coli* ($r = 0.91779$), *A. niger* ($r = 0.956461$), and *C. albicans* ($r = 0.937803$). A comparatively weaker correlation was observed for *B. spizizenii* ($r = 0.69611$), while *P. aeruginosa* showed a moderate correlation ($r = 0.844924$). Additionally, strong interspecies correlations were recorded, particularly between *S. mutans* and *E. coli* ($r =$

0.991449), and between *A. niger* and *C. albicans* ($r = 0.99753$), indicating similar response patterns across microbial groups. Overall, the results demonstrate a consistent, concentration-dependent increase in antimicrobial activity of *C. aureus* muscle extracts, with closely related inhibition trends across tested microorganisms.

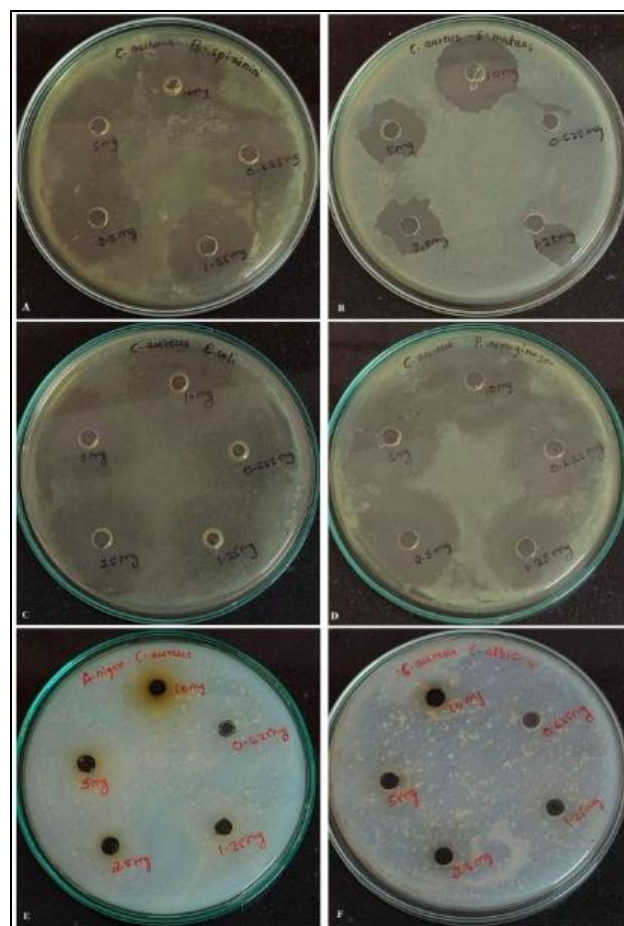


Figure 1: Antimicrobial activity of *Chrysochir aureus* muscle extracts. Inhibitory zones against A) *B. spizizenii* B) *S. mutans* C) *E. coli* D) *P. aeruginosa* E) *A. niger* F) *C. albicans*

Table 13: Antimicrobial activity of *Chrysochir aureus* muscle extracts with increasing concentration

Name of the Organism			Diameter of the Inhibition Zone (mm)					Positive Control (Erythromycin)	Negative Control (DMSO)
			Concentration of compound (mg/ml)						
			0.625	1.25	2.5	5.0	10		
Gram Positive Bacteria	<i>Bacillus spizizenii</i>	21	29	31	32	33	33	-	
	<i>Streptococcus mutans</i>	16	20	24	25	32	32	-	
Gram Negative Bacteria	<i>Escherichia coli</i>	22	26	28	29	33	33	-	
	<i>Pseudomonas aeruginosa</i>	27	28	32	33	34	26	-	
Fungi	<i>Aspergillus niger</i>	-	-	-	10	15	18	-	
	<i>Candida albicans</i>	-	-	-	9	12	25	-	

Table 14: Correlation analysis of the antimicrobial activity of *Chrysochir aureus* muscle extracts with increasing concentration

Microbial Species	Ex. Conc.	<i>B. spizizenii</i>	<i>S. mutans</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>A. niger</i>	<i>C. albicans</i>
<i>B. spizizenii</i>	0.69611	1					
<i>S. mutans</i>	0.94949	0.864	1				
<i>E. coli</i>	0.91779	0.917911	0.991449	1			
<i>P. aeruginosa</i>	0.844924	0.869923	0.931048	0.926502	1		
<i>A. niger</i>	0.956461	0.623921	0.856804	0.831926	0.794634	1	
<i>C. albicans</i>	0.937803	0.628371	0.84022	0.819547	0.798848	0.99753	1

Antimicrobial activity of *Otolithes ruber*:

The muscle extracts of *Otolithes ruber* exhibited maximum antimicrobial activity at 10 mg/ml against all tested microorganisms. The highest inhibition was observed against *P. aeruginosa* (33 mm), followed by *B. spizizenii* (31 mm), *S. mutans* (30 mm), *E. coli* (28 mm), *C. albicans* (16 mm), and *A. niger* (10 mm). At 0.625 mg/ml, the extracts showed antibacterial activity against *B. spizizenii* (28 mm), *P. aeruginosa* (27 mm), *E. coli* (24 mm), and *S. mutans* (16 mm). Antifungal activity against *A. niger* and *C. albicans* was detected only at 5 and 10 mg/ml, whereas no activity was observed at lower concentrations. The concentration-dependent antimicrobial activity is presented in Table 15 and Figure 2.

Correlation analysis (Table 16) showed a strong positive relationship between extract concentration and antimicrobial activity against all tested microorganisms, including *B. spizizenii* ($r = 0.968266$), *S. mutans* ($r = 0.981882$), *E. coli* ($r = 0.933257$), *P. aeruginosa* ($r = 0.937460$), *A. niger* ($r = 0.898214$), and *C. albicans* ($r = 0.974040$). High positive correlations were also observed among microbial responses, indicating consistent inhibition patterns across species. These findings demonstrate that the antimicrobial activity of *O. ruber* muscle extracts is concentration-dependent and exhibits broad-spectrum antimicrobial potential.

Table 15: Antimicrobial activity of *Otolithes ruber* muscle extracts with increasing concentration

Name of the Organism		Diameter of the Inhibition Zone (mm)						
		Concentration of compound (mg/ml)					Positive Control (Erythromycin)	Negative Control (DMSO)
		0.625	1.25	2.5	5.0	10		
Gram Positive Bacteria	<i>Bacillus spizizenii</i>	28	28	29	30	31	33	-
	<i>Streptococcus mutans</i>	16	18	20	21	30	32	-
Gram Negative Bacteria	<i>Escherichia coli</i>	24	25	26	27	28	33	-
	<i>Pseudomonas aeruginosa</i>	27	28	29	32	33	26	-
Fungi	<i>Aspergillus niger</i>	-	-	-	-	10	18	-
	<i>Candida albicans</i>	-	-	-	9	16	25	-

Table 16: Correlation analysis of the antimicrobial activity of *Otolithes ruber* muscle extracts with increasing concentration

Microbial Species	Ex. Conc.	<i>B. spizizenii</i>	<i>S. mutans</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>A. niger</i>	<i>C. albicans</i>
<i>B. spizizenii</i>	0.968266	1					
<i>S. mutans</i>	0.981882	0.925742	1				
<i>E. coli</i>	0.933257	0.970143	0.910191	1			
<i>P. aeruginosa</i>	0.93746	0.977805	0.87882	0.977356	1		
<i>A. niger</i>	0.898214	0.771744	0.934262	0.707107	0.691095	1	
<i>C. albicans</i>	0.97404	0.948157	0.91826	0.890463	0.94194	0.844656	1

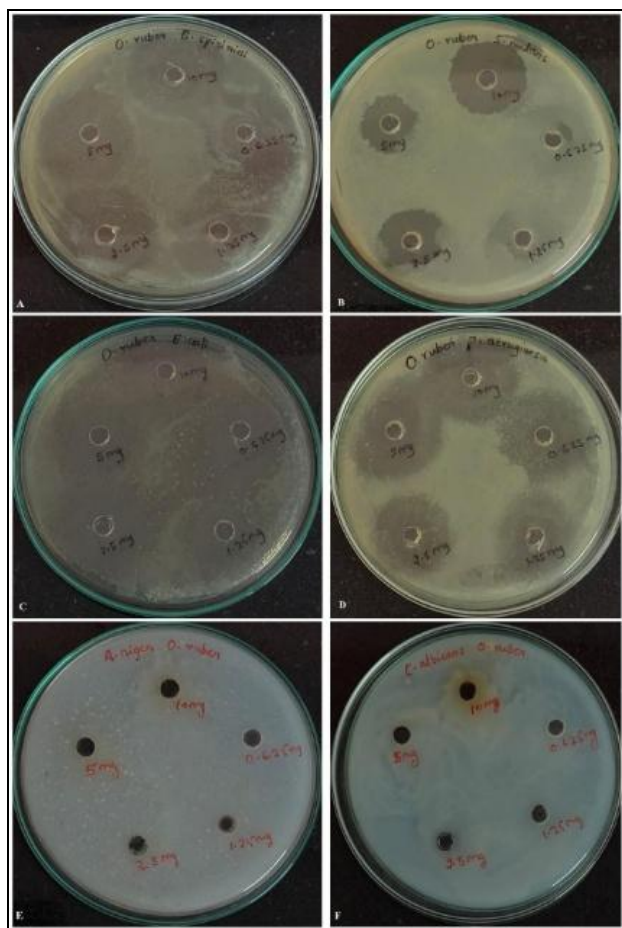


Figure 2: Antimicrobial activity of *Otolithes ruber* muscle extracts. Inhibitory zones against A) *B. spizizenii* B) *S. mutans* C) *E. coli* D) *P. aeruginosa* E) *A. niger* F) *C. albicans*

DISCUSSION

The present study demonstrated that the chloroform–methanol muscle extracts of *C. aureus* and *O. ruber* possess significant antioxidant and antimicrobial activities, highlighting their potential as natural sources of bioactive compounds. Both extracts exhibited concentration-dependent antioxidant activity in DPPH, ABTS, FRAP, superoxide, nitric oxide, and total antioxidant capacity (TAC) assays, indicating their ability to neutralize reactive oxygen and nitrogen species through hydrogen- or electron-donating mechanisms. These activities are likely associated with bioactive peptides, amino acids, phenolic compounds, and other antioxidant metabolites extracted by the chloroform–methanol solvent system (Krishnaraju et al., 2009; Ngo et al., 2012).

The antioxidant activities observed are consistent with previous studies on marine fish extracts and protein

hydrolysates, which reported concentration-dependent radical scavenging activity and reducing power (Nagai et al., 2006; Duh et al., 1999; Samaranayaka and Li-Chan, 2011; Zhao et al., 2019; Noman et al., 2022). The relatively higher TAC of *O. ruber* at lower concentrations may reflect species-specific differences in muscle biochemical composition.

Both fish extracts also exhibited broad-spectrum antimicrobial activity, with stronger inhibition against bacterial pathogens than fungal species. The highest activity was observed against *Pseudomonas aeruginosa*, while antifungal activity was detected only at higher extract concentrations. These findings agree with earlier reports attributing the antimicrobial properties of marine fish tissues to antimicrobial peptides and proteins that disrupt microbial cell membranes and inhibit pathogen growth (Moore et al., 1993; Lauth et al., 2002; Gomez et al., 2010).

Overall, the results indicate that *C. aureus* and *O. ruber* muscle extracts are promising sources of natural antioxidant and antimicrobial compounds with potential applications in functional foods, nutraceuticals, pharmaceuticals, and natural food preservation. Further studies are needed to isolate and characterize the active constituents and to confirm their efficacy and safety through *in vivo* and toxicity evaluations.

CONCLUSION

The present study concludes that the chloroform–methanol muscle extracts of *Chrysochir aureus* and *Otolithes ruber* are promising natural sources of bioactive compounds with notable antioxidant and antimicrobial properties. The concentration-dependent radical scavenging and reducing activities observed across multiple *in vitro* assays (DPPH, ABTS, FRAP, superoxide, nitric oxide, and TAC) indicate the presence of effective hydrogen- and electron-donating constituents, likely including bioactive peptides and other secondary metabolites derived from fish muscle. In addition, the demonstrated antibacterial activity, greater than antifungal effects, suggests the presence of antimicrobial peptides capable of disrupting microbial growth, consistent with the innate immune components of marine organisms. The overall results highlight species-specific differences in bioactivity, particularly the comparatively higher antioxidant

potential observed in *O. ruber* at lower concentrations. These findings support the potential application of both fish species as alternative natural sources for the development of nutraceuticals, functional food ingredients, pharmaceutical agents, and natural preservatives. Nevertheless, further work involving purification, structural characterization of active compounds, and *in vivo* validation, including toxicity studies, is necessary to fully establish their therapeutic and commercial potential.

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Conflict of interest

The authors declare no competing interests.

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