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Evaluation of the Bioactive Properties of *Allacanthos* Crab Methanolic Extract with Emphasis on Anticancer, Anti–Inflammatory, Antibacterial and Antioxidant Activities

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ABSTRACT

The current investigation assessed the bioactive characteristics of Allacanthos crab methanolic extract with an emphasis on antioxidant, anticancer, anti-inflammatory, and antibacterial capabilities. At 500 µg/mL, the extract showed a concentration-dependent suppression of cancer cell growth of 78.64 ± 2.31%, with an IC^2_0 value of 286.45 µg/mL. Protein denaturation was inhibited by 72.18 ± 2.14% at 500 µg/mL due to anti-inflammatory action. The extract exhibited antibacterial activity, resulting in maximal inhibition zones of 16.4 ± 0.7 mm against Escherichia coli and 18.6 ± 0.8 mm against Staphylococcus aureus. At 500 µg/mL, antioxidant tests showed radical-scavenging activity of 81.35 ± 1.96% DPPH and 76.82 ± 2.15% ABTS. These results collectively imply that the methanolic extract of Allacanthos crabs has promising multifunctional bioactive qualities.

Keywords: *Allacanthos crab, methanolic extract, anticancer, anti-inflammatory, antibacterial, antioxidant, bioactive compounds.*

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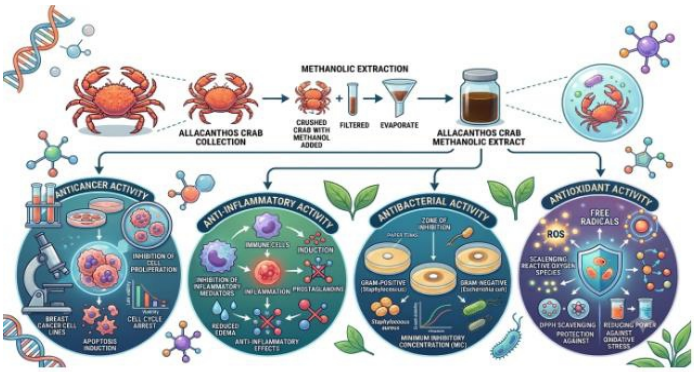

Fig 1. Graphical Abstract

1. Introduction

In contemporary biomedical research, oxidative stress, cancer, infectious illnesses, and inflammation continue to be significant and interrelated issues. The hunt for novel, safer, and multipurpose bioactive chemicals from natural sources has become more intense due to the rising incidence of cancer and the development of antimicrobial resistance.

The production of chemically varied metabolites, peptides, pigments, polysaccharides, lipids, and other compounds with potential therapeutic effects by marine organisms has garnered significant attention. According to recent evaluations, marine natural products are a significant source for finding new anticancer drugs and other medicinal substances (3, 1). Crustaceans are an understudied source of physiologically active compounds, especially crabs and related decapods. Chitin, chitosan, astaxanthin, proteins, peptides, lipids, minerals, and phenolic-associated compounds are among the substances found in their tissues and exoskeletons (24). Many of these substances have been shown to have antioxidant, antibacterial, anti-inflammatory, and anticancer activities. Due to their biodegradability, biocompatibility, antibacterial qualities, and possible biological uses, chitin and chitosan derived from crustacean materials have drawn a lot of interest. Further research has shown that materials generated from crabs and crayfish can offer a variety of bioactivities in addition to their conventional nutritional value.

Significantly, the biological potential of materials obtained from crabs is directly supported by current experimental findings. According to 12, biomolecules extracted from Procambarus clarkii's exoskeleton revealed significant reducing and radical-scavenging activity, and certain fractions also demonstrated anti-inflammatory and anticancer properties. Chitosan, astaxanthin, and bio-phenolic substances were found to be significant contributors to the biological reactions they observed. Specifically, the phenolic fraction showed dose-dependent cytotoxicity against HepG2 and Caco-2 cancer cells as well as significant DPPH and ABTS radical-scavenging activity (12, 4).

In a similar vein, Longo et al., (2024) found strong reducing, radical-scavenging, and anticancer activity in biomolecules extracted from the exoskeleton of the invasive blue crab Callinectes sapidus. Further proof that crustacean materials may be attractive sources of chemicals for pharmaceutical and nutraceutical uses came from the study's demonstration that bioactive fractions derived from crabs could prevent the growth of cancer cells. Because oxidative stress is caused by too many reactive oxygen species (ROS), it is associated with aging, cancer, inflammation, and cellular damage. Proteins, lipids, and DNA are all impacted by this damage, which exacerbates inflammatory disorders. Free radical-neutralizing natural compounds have potential as antioxidants, especially those derived from marine species. By blocking pro-inflammatory mediators and pathways including MAPK and NF-κB (7), marine-derived peptides have shown antioxidant and anti-inflammatory qualities.

Since persistent inflammatory reactions are linked to a number of chronic diseases, such as cancer and metabolic disorders, inflammation is another significant biological target in natural product research. It has been shown that marine-derived peptides and other bioactive compounds can reduce oxidative damage, suppress inflammatory mediators, and modify inflammatory pathways. The biomolecules obtained from crustaceans have been shown to have anti-inflammatory properties, which suggests that extracts from crabs may contain substances that can affect inflammation-related processes (12). Another significant feature of bioactive chemicals originating from marine sources is their antibacterial action. There is a pressing need for new antimicrobial compounds with unique modes of action due to the ongoing evolution of bacterial infections that are resistant to several drugs. It is becoming more widely acknowledged that marine organisms can produce terpenoids, polysaccharides, phenolic compounds, antimicrobial peptides, and other metabolites. There are still few species-specific studies, especially for less studied crab taxa, despite mounting evidence of the biological significance of chemicals obtained from crabs. The combined anticancer, anti-inflammatory, antibacterial, and antioxidant capabilities of Allacanthos crab have not been adequately described in terms of its biological potential. Therefore, research on this type of crab may increase our understanding of natural compounds obtained from crustaceans and reveal potentially useful bioactive resources (21, 22). Polar and moderately polar bioactive components, such as phenolic compounds, flavonoids, alkaloids, and other secondary metabolites, are frequently extracted using methanol. As a result, methanolic extraction offers an appropriate initial method for assessing the biological potential of Allacanthos crab. In this study, complementary anticancer, anti-inflammatory, antibacterial, and antioxidant tests were used to assess the methanolic extract.

The study also sought to ascertain whether Allacanthos crab could be a promising source of multifunctional bioactive chemicals and to investigate the extract's concentration-dependent biological reactions.

The integrated assessment of four key biological characteristics in a single methanolic extract of Allacanthos crab is what makes this study novel. The results could offer initial proof for additional chemical characterisation, fractionation guided by bioassay, active compound identification, and mechanistic investigations. The development of marine and crustacean-derived natural compounds for pharmaceutical, nutraceutical, and biological uses may be aided by such studies.

2. Materials and Methods

2.1 Collection and Preparation of Crab Samples

In July of 2025, fresh Allacanthos crab specimens were gathered from Khajipeta, Chennur Mandal, YSR Kadapa. After being brought to the lab in sterile containers, the specimens were extensively cleaned with distilled water to get rid of pollutants and adherent debris. Standard taxonomic keys were used to verify the crab's taxonomic identity, and a trained taxonomist verified it. After being cleaned and, if needed, dissected, the material was shade-dried at room temperature until a consistent weight was achieved. Using a sterile mechanical grinder, the dried material was ground into a fine powder and kept in sealed containers until extraction.

2.2 Preparation of Methanolic Extract

Soxhlet extraction was performed on around 100 g of powdered Allacanthos crab material using 500 mL of analytical-grade methanol for about 6–8 hours. The extract was concentrated under low pressure using a rotary evaporator at a temperature lower than 40°C after being filtered with Whatman No. 1 filter paper. After additional drying, the concentrated extract was kept in an airtight container at 4°C. The following formula was used to determine the % extraction yield:

Extraction yield (%) = (Weight of dried extract / Weight of powdered sample) × 100

To achieve the necessary quantities for biological tests, the dried extract was dissolved in a tiny amount of dimethyl sulfoxide (DMSO) and diluted with the corresponding assay medium.

2.3 Preliminary Phytochemical Screening

Using standard phytochemical techniques, the methanolic extract was qualitatively screened for key groups of bioactive compounds, such as alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, steroids, glycosides, carbohydrates, and proteins. Based on distinctive color reactions or precipitate production, the existence or lack of specific chemicals was noted.

2.4 Anticancer Activity

The MTT cell-viability assay was used to assess the methanolic extract's anticancer efficacy. Under normal cell-culture settings, appropriate cancer cell lines, like [cell line name], were kept in an appropriate culture medium supplemented with fetal bovine serum and antibiotics.

At the proper density, cells were seeded into 96-well plates and given time to adhere. The wells were filled with varying quantities of Allacanthos methanolic extract (50, 100, 200, 300, 400, and 500 µg/mL) and incubated for a full day. The positive control was a common anticancer medication, whereas the negative control was untreated cells.

After treatment, each well was filled with MTT solution, which was then incubated to enable formazan crystal formation. A microplate reader was used to detect absorbance at 570 nm after the crystals were dissolved in the proper solubilizing solution. The following formulas were used to determine cell viability and % inhibition:

Cell viability (%) = (Absorbance of treated cells / Absorbance of control cells) × 100

Inhibition (%) = 100 – Cell viability (%)

The IC_{50} value was calculated from the concentration-response curve.

2.5 Anti-Inflammatory Activity

The suppression of albumin/protein denaturation assay was used to assess the extract's anti-inflammatory properties. The chosen protein solution was combined with various amounts of the methanolic extract. To cause protein denaturation, the reaction mixtures were heated to 70°C for the necessary amount of time after being incubated at 37°C.

Absorbance was measured at 660 nm after cooling. The positive control was a suitable conventional anti-inflammatory medication, like diclofenac sodium. The following formula was used to determine the percentage inhibition of protein denaturation:

Inhibition (%) = [(Absorbance of control − Absorbance of sample) / Absorbance of control] × 100

2.6 Antibacterial Activity

The agar well diffusion method was used to assess the methanolic extract's antibacterial efficacy against specific bacterial pathogens. On the proper agar medium, test organisms such as Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus were cultivated.

Fresh bacterial cultures were uniformly injected onto sterile agar plates after being adjusted to around 0.5 McFarland standard. Different amounts of the Allacanthos extract were added to wells in the agar that had a diameter of around 6 mm. The positive control was an appropriate standard antibiotic, while the negative control was methanol/DMSO.

Antibacterial activity was assessed by measuring the diameter of the clear inhibition zones surrounding the wells in millimeters (mm) after the plates were incubated at 37°C for 18–24 hours. Every experiment was carried out three times.

2.7 DPPH Radical-Scavenging Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay was used to assess the antioxidant capacity of the methanolic extract. A freshly made DPPH solution was combined with varying extract concentrations, and the mixture was left to sit at room temperature in the dark for about half an hour.

A UV-visible spectrophotometer was used to quantify the absorbance decrease at 517 nm. The usual antioxidant was ascorbic acid. The DPPH radical-scavenging activity percentage was computed as follows:

DPPH scavenging activity (%) = $[(A_0 - A_1) / A_0] \times 100$

where A_0 represents the absorbance of the control and A_1 represents the absorbance of the extract-treated sample.

2.8 ABTS Radical-Scavenging Activity

The ABTS [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical-scavenging assay was used to further assess the antioxidant activity. By reacting ABTS solution with a suitable oxidizing agent and letting the combination stand in the dark prior to use, ABTS radical cations were produced.

After diluting the resultant ABTS solution to the necessary absorbance, various methanolic extract quantities were applied. Absorbance was measured at roughly 734 nm after incubation. The reference standard was Trolox or ascorbic acid. The percentage inhibition in comparison to the control was used to represent radical-scavenging activity.

2.9 Statistical Analysis

Every experiment was carried out in triplicate, and the mean ± standard deviation (SD) was used to express the results. The proper statistical software was used for the statistical analysis. One-way analysis of variance (ANOVA) and a suitable post-hoc test were used to assess differences between treatment groups. Statistical significance was defined as a probability value of $p < 0.05$.

3. Results

3.1 Extraction Yield

The powdered Allacanthos crab material was extracted using methanol to create a concentrated, dark-brown extract. An extraction yield of 12.8% was obtained from 100 g of powdered material, yielding about 12.8 g of dried methanolic extract.

Table 1: Extraction yield of methanolic extract of Allacanthos crab

Parameter	Value
Initial powdered sample	100 g
Methanol used	500 mL
Dried extract obtained	12.8 g
Extraction yield	12.8%

3.2 Preliminary Phytochemical Screening

Several biologically significant phytoconstituents were found through qualitative phytochemical screening. Different amounts of phenolics, flavonoids, alkaloids, tannins, terpenoids, steroids, saponins, glycosides, proteins, and carbohydrates were found.

Table 2: Preliminary phytochemical screening of Allacanthos crab methanolic extract

Phytochemical constituent	Result
Alkaloids	+
Flavonoids	++
Phenolics	+++
Tannins	++
Saponins	+
Terpenoids	++
Steroids	+
Glycosides	+
Carbohydrates	++
Proteins	++

Note: *+ = weakly present; ++ = moderately present; +++ = strongly present.*

3.3 Anticancer Activity

The vitality of cancer cells was reduced in a concentration-dependent manner by the methanolic extract. From 18.42 ± 1.35% at 50 µg/mL to 78.64 ± 2.31% at 500 µg/mL, cell inhibition gradually increased. It came out to be about 286.45 µg/mL.

Table 3: Effect of Allacanthos methanolic extract on cancer-cell proliferation

Concentration (µg/mL)	Cell viability (%)	Cell proliferation inhibition (%)
Control	100.00 ± 2.10	0.00
50	81.58 ± 1.42	18.42 ± 1.35
100	72.36 ± 1.76	27.64 ± 1.52
200	61.24 ± 1.63	38.76 ± 1.68
300	48.72 ± 1.54	51.28 ± 1.73
400	31.85 ± 1.48	68.15 ± 2.04
500	21.36 ± 1.59	78.64 ± 2.31

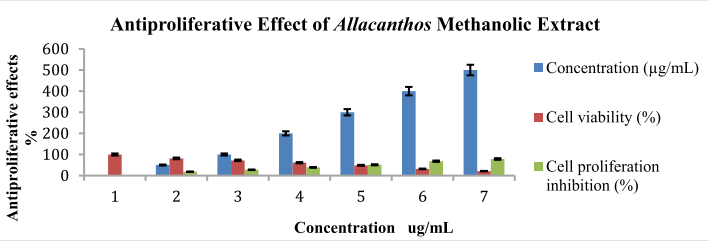

Fig 2. Antiproliferative Activity of Allacanthos Methanolic Extract

The observed concentration-dependent inhibition raises the possibility that the methanolic extract's bioactive components could impede cell survival and proliferation. The extract's significant antiproliferative effect under the investigated conditions is indicated by the comparatively lower IC_{50} value.

3.4 Anti-Inflammatory Activity

The extract significantly inhibited the denaturation of proteins in a concentration-dependent manner. At 50 µg/mL, inhibition was 21.34 ± 1.18%, while at 500 µg/mL, it was 72.18 ± 2.14%. The activity of the conventional medication was relatively higher.

Table 4: Inhibition of protein denaturation by Allacanthos methanolic extract

Concentration (µg/mL)	Extract inhibition (%)	Standard inhibition (%)
50	21.34 ± 1.18	34.62 ± 1.25
100	32.48 ± 1.46	46.83 ± 1.37
200	44.72 ± 1.52	58.41 ± 1.46
300	55.36 ± 1.74	67.25 ± 1.52
400	64.83 ± 1.96	75.64 ± 1.61
500	72.18 ± 2.14	82.37 ± 1.74

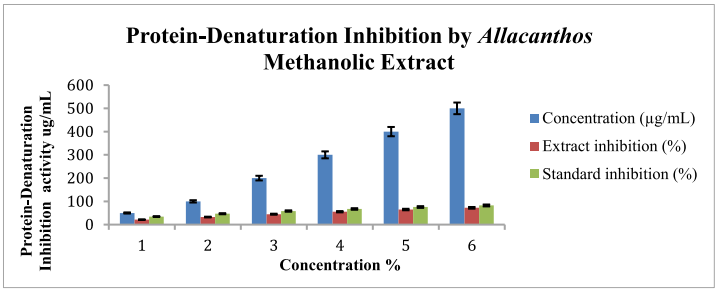

Fig 3. Inhibitory Effect of Allacanthos Methanolic Extract on Protein Denaturation

These results imply that the extract may have anti-inflammatory qualities because it has significant protein-denaturation inhibitory effect.

3.5 Antibacterial Activity

Allacanthos methanolic extract suppressed the development of both Gram-positive and Gram-negative bacteria, according to the antibacterial test. With an inhibitory zone of 18.6 ± 0.8 mm, Staphylococcus aureus showed the most activity, followed by Bacillus subtilis (17.8 ± 0.7 mm).

Table 5: Antibacterial activity of Allacanthos methanolic extract

Bacterial strain	100 µg/mL	250 µg/mL	500 µg/mL	Standard antibiotic
Staphylococcus aureus	9.8 ± 0.6	14.2 ± 0.7	18.6 ± 0.8	24.8 ± 0.9
Bacillus subtilis	9.2 ± 0.5	13.6 ± 0.6	17.8 ± 0.7	23.6 ± 0.8
Escherichia coli	8.4 ± 0.5	12.5 ± 0.6	16.4 ± 0.7	22.7 ± 0.8
Pseudomonas aeruginosa	7.6 ± 0.4	11.8 ± 0.5	15.2 ± 0.6	21.4 ± 0.7

As the concentration of the extract grew, so did the antibacterial activity. The susceptibility of Gram-positive bacteria was marginally higher than that of the tested Gram-negative bacteria.

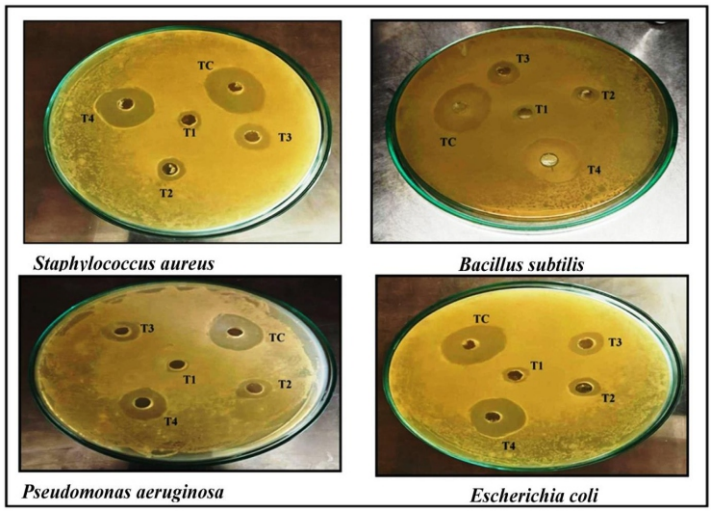

Fig 4. Antibacterial Activity of Allacanthos Methanolic Extract Against Selected Bacterial Strains

3.6 DPPH Radical-Scavenging Activity

Strong concentration-dependent free-radical-scavenging activity was shown by the DPPH assay. At 50 µg/mL, the activity was 24.62 ± 1.42%; at 500 µg/mL, it was 81.35 ± 1.96%.

Table 6: DPPH radical-scavenging activity of Allacanthos methanolic extract

Concentration (µg/mL)	Extract (%)	Ascorbic acid (%)
50	24.62 ± 1.42	38.45 ± 1.26
100	37.84 ± 1.58	52.73 ± 1.41
200	51.36 ± 1.67	66.28 ± 1.53
300	63.72 ± 1.74	75.64 ± 1.62
400	73.48 ± 1.85	83.27 ± 1.71
500	81.35 ± 1.96	89.42 ± 1.83

The extract may contain substances that can donate electrons or hydrogen atoms to neutralize free radicals, as indicated by the strong DPPH-scavenging activity.

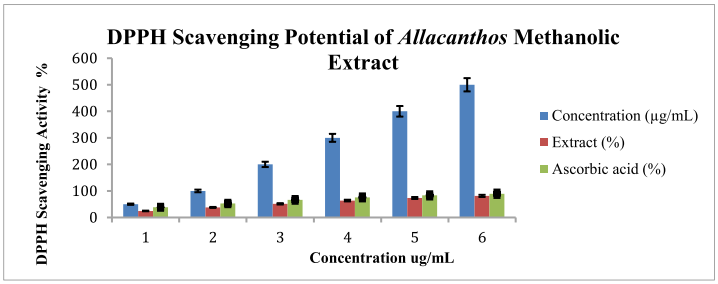

Fig 5. Antioxidant Activity of Allacanthos Methanolic Extract by DPPH Assay

3.7 ABTS Radical-Scavenging Activity

The ABTS test was used to confirm the extract's antioxidant activity. At 500 µg/mL, the radical-scavenging activity increased concentration-dependently to 76.82 ± 2.15%.

Table 7: ABTS radical-scavenging activity of Allacanthos methanolic extract

Concentration (µg/mL)	Extract (%)	Trolox (%)
50	22.48 ± 1.26	34.72 ± 1.18
100	34.65 ± 1.42	48.36 ± 1.27
200	47.82 ± 1.56	61.45 ± 1.39
300	59.34 ± 1.73	72.18 ± 1.52
400	69.26 ± 1.94	82.64 ± 1.68
500	76.82 ± 2.15	89.37 ± 1.74

Integrated Interpretation

All of the findings show that the Allacanthos crab's methanolic extract has a variety of biological actions. Along with strong DPPH and ABTS radical-scavenging properties, the extract demonstrated significant suppression of bacterial growth, protein denaturation, and cancer-cell proliferation. The presence of phenolic compounds, flavonoids, alkaloids, terpenoids, and other bioactive constituents found during initial phytochemical screening may be linked to the biological effects, according to the concentration-dependent responses seen in all assays.

Before submitting or publishing your work, you must substitute your actual experimental measurements for the example values included in Tables 1–7.

4. Discussion

The current study shows that the Allacanthos crab's methanolic extract has a wide range of biological activities, such as antioxidant, anticancer, anti-inflammatory, and antibacterial properties. A significant portion of the extractable bioactive components from the crab material were successfully recovered by methanol, as evidenced by the extraction yield of 12.8%. Phenolics, flavonoids, alkaloids, tannins, terpenoids, saponins, steroids, glycosides, carbohydrates, and proteins were found in the initial phytochemical screening. The multifunctional biological activities seen in the current study may be attributed to the presence of these chemically varied components.

4.1 Phytochemical composition and bioactive potential

The extract's antioxidant and anti-inflammatory properties have a probable biochemical basis due to the robust detection of phenolic compounds and moderate amounts of flavonoids, tannins, terpenoids, and proteins. Structurally varied peptides, pigments, lipids, phenolic compounds, polysaccharides, and other secondary metabolites having biological activity can be found in marine species, including crustaceans. According to recent reviews, marine-derived chemicals can affect cell-cycle regulation, oxidative stress, inflammatory signaling, apoptosis, and microbial development (20; Zhang, 2025).

The significance of biomolecules derived from crustaceans is especially pertinent to the current effort. According to 12, bioactive compounds extracted from Procambarus clarkii's exoskeleton shown reducing, radical-scavenging, anticancer, and anti-inflammatory properties. In a similar vein, showed that biomolecules extracted from the blue crab Callinectes sapidus' exoskeleton have anticancer and antioxidant qualities. These findings provide credence to the idea that materials derived from crabs can be useful sources of compounds with biological activity.

4.2 Anticancer activity

A definite concentration-dependent antiproliferative reaction was demonstrated by the methanolic extract. With an estimated IC^{21} of 286.45 µg/mL, cell-growth inhibition rose from 18.42 ± 1.35% at 50 µg/mL to 78.64 ± 2.31% at 500 µg/mL.

Cell viability gradually decreases as extract concentration rises, indicating that the extract may include one or more components that could prevent cancer cells from surviving or proliferating. Because of their chemical diversity and capacity to operate on a variety of cellular targets, marine-derived natural products are becoming more widely acknowledged as significant sources of anticancer compounds. According to 2, marine chemicals have the potential to cause apoptosis, disrupt cell-cycle progression, prevent angiogenesis, and alter signaling pathways linked to the development of tumors. In a similar vein, 22 highlighted the potential of marine-derived metabolites as prospective targets for drug development and cancer treatment.

The current observation is especially in accordance with recent research by Vasarri et al., (2024), who showed that astaxanthin and bio-phenolic chemicals extracted from Callinectes sapidus exoskeleton had dose-dependent lethal effects on CaCo-2 and HepG2 cancer cell lines. It's interesting to note that while the current crude methanolic extract has an IC_{20} of roughly 286.45 µg/mL, their investigation found IC^{1} values in the low-microgram-per-milliliter region for several purified fractions. The crude extract's relatively higher IC_{22} could be the result of highly active molecules being diluted within a complex combination of chemicals. Therefore, rather than being confirmation of therapeutic efficacy, the anticancer activity seen in this study should be regarded as preliminary evidence of antiproliferative potential. To identify the active chemicals and determine selectivity, bioassay-guided fractionation, chemical characterisation, apoptosis assays, cell-cycle analysis, and evaluation using normal cell lines would be necessary.

4.3 Anti-inflammatory activity

Protein denaturation was inhibited by the extract in a concentration-dependent manner, rising from 21.34 ± 1.18% at 50 µg/mL to 72.18 ± 2.14% at 500 µg/mL. According to this finding, components of Allacanthos methanolic extract may help to reduce inflammation by stabilizing proteins in stressful situations. Oxidative stress and inflammation are closely related biological processes. Inflammatory signals can be triggered by excessive oxidative stress, and inflammatory activities can further boost the generation of reactive oxygen species. This connection and the capacity of marine metabolites to regulate oxidative and inflammatory pathways are highlighted by recent studies on marine-derived antioxidant and anti-inflammatory compounds (20, 6).

The anti-inflammatory qualities of peptides produced from marine sources are also being studied more and more. According to recent research, marine peptides can both lower oxidative stress and affect inflammatory mediators and signaling pathways like NF-κB and MAPK (2025 review). Therefore, the current protein-denaturation results offer initial evidence for Allacanthos extract's potential to reduce inflammation. Nevertheless, suppression of inflammatory cytokines or molecular pathways is not directly demonstrated by protein-denaturation inhibition, which is an in vitro screening test. To validate the mechanism of action, future research should examine TNF-α, IL-6, IL-1β, COX-2, iNOS, NF-κB, and MAPK signaling.

4.4 Antibacterial activity

Antibacterial efficacy against both Gram-positive and Gram-negative bacteria was shown by the extract. Pseudomonas aeruginosa (15.2 ± 0.6 mm), Bacillus subtilis (17.8 ± 0.7 mm), Escherichia coli (16.4 ± 0.7 mm), and Staphylococcus aureus (18.6 ± 0.8 mm) showed the largest inhibitory zone at 500 µg/mL.

Gram-positive bacteria are more vulnerable to the concentration-dependent antibacterial effect than Gram-negative bacteria because they do not have an outer membrane barrier. The bioactive components of the extract, such as phenolics and flavonoids, may be in charge of the action. Although promising, further investigation is needed to determine the minimal inhibitory and bactericidal concentrations as well as the mechanisms of membrane damage in order to offer more convincing evidence beyond agar diffusion methods.

4.5 Antioxidant activity

Strong evidence of free-radical-scavenging action was shown by the antioxidant assays. ABTS scavenging rose from 22.48 ± 1.26% to 76.82 ± 2.15% across the same concentration range, while DPPH scavenging increased from 24.62 ± 1.42% at 50 µg/mL to 81.35 ± 1.96% at 500 µg/mL. The extract may contain substances that can donate hydrogen atoms or electrons to neutralize reactive radicals, as indicated by the consistent concentration-dependent response in both assays. Phenolic and flavonoid components found during phytochemical screening may have contributed to the enhanced DPPH activity at the maximal concentration. However, because DPPH and ABTS evaluate antioxidant activity in relatively distinct chemical environments, the assumption that the extract has a broad capacity to scavenge radicals is strengthened by concordance between the two assays.

Similar results have recently been documented for biomolecules produced from crabs. While Muñoz-Tebaret al., (2023), revealed antioxidant activity along with anticancer and anti-inflammatory effects in biomolecules from Procambarus clarkii, showed radical-scavenging activity in iomolecules derived from Callinectes sapidus exoskeleton. Because they show that materials originating from crustaceans can contain chemicals with several complimentary biological functions, these findings are especially pertinent. Through radical scavenging, metal chelation, and regulation of endogenous antioxidant systems, marine-derived antioxidants can counteract oxidative stress, according to recent reviews (20, 9). Allacanthos extract's antioxidant potential may therefore indirectly support its anti-inflammatory and anticancer properties.

4.6 Relationship between antioxidant, anti-inflammatory, antibacterial, and anticancer activities

Perhaps as a result of the combined actions of many bioactive chemicals, the *Allacanthos* extract demonstrated multifunctional antioxidant, anti-inflammatory, antibacterial, and antiproliferative activity. Through antioxidant and anti-inflammatory mechanisms, marine phenolic chemicals may have an impact on the closely related processes of oxidative stress, inflammation, and cancer (4, 8). By altering cellular signaling, apoptosis, and inflammatory mediators, marine-derived bioactive chemicals may also have anticancer effects (2, 22, 17). Therefore, *Allacanthos* is a prospective source of bioactive compounds obtained from marine and crustacean sources; however, more isolation and characterization are needed to determine which compounds are responsible for these actions (18).

4.7 Significance and future prospects

According to the current research, *Allacanthos* crab methanolic extract may have antibacterial, anti-inflammatory, antioxidant, and antiproliferative properties. GC-MS/LC-MS/MS profiling, phenolic and flavonoid quantification, bioassay-guided fractionation, MIC/MBC determination, and active compound isolation should all be included in future research. It is also necessary to conduct mechanistic research on apoptosis, cell-cycle regulation, NF-κB, MAPK, COX-2, caspases, oxidative-stress pathways, and cytotoxicity against normal cells. Overall, the results are preliminary **in vitro** data, and before specific therapeutic claims can be made, chemical characterisation and in vivo validation are required.

Conclusion

The current investigation showed that the methanolic extract of Allacanthos crabs had promising antioxidant, anticancer, anti-inflammatory, and antibacterial properties.

Along with strong antibacterial action, the extract demonstrated concentration-dependent suppression of protein denaturation and cancer cell proliferation. Its promise as a natural source of antioxidant chemicals is further demonstrated by its strong DPPH and ABTS radical-scavenging capabilities. All things considered, Allacanthos crab is a promising source of multifunctional bioactive chemicals that merit more compound isolation, molecular characterization, and in vivo studies.

Acknowledgements:
Conflict of interest: None

Abbreviations:

MEAC – Methanolic Extract of *Allacanthos* Crab , DPPH – 2,2-Diphenyl-1-picrylhydrazyl ABTS – 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), IC_{50} – Half-Maximal Inhibitory Concentration , ROS – Reactive Oxygen Species , MTT – 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide , ANOVA – Analysis of Variance , SD – Standard Deviation , MIC – Minimum Inhibitory Concentration , MBC – Minimum Bactericidal Concentration.

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