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Research Article

Muricidae Gastropod Operculum Extract as a Unique Agent against the Osteosarcoma Cell Line: Unveiling its Anti-Angiogenic and Antioxidant Potential

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ABSTRACT

Aim: India exports the marine Gastropod operculum, which is used in herbal medicine in Indian communities. **Background:** Because of its usage in traditional medicine, the operculum provides a source of bioactive chemicals. Free radical scavenging activity, reducing power movement, and H_2O_2 radical scavenging activity all contributed to the antioxidant potential. The anticancer compounds in clinical characteristics are particularly well represented by natural chemicals extracted from molluscs and their structural counterparts. **Materials and Methods:** The gastropod protein extract provides several benefits as a prospective material for anticancer treatments with no adverse effects. The pharmacodynamic effects of a chloroform extract from *Chicoreus ramosus* were explored in cell lines through the Mossman technique for cytotoxicity assay. **Results:** The cell viability of MG-63 cells ranged from 31.33% to 99.73% at a dosage of 500 µg/mL. The anti-inflammatory properties and processes of action may be linked to flavonoids, which are known to prevent membrane lysis, albumin denaturation, and protease activity. The maximal dose of 100 g/mL inhibited branching points and reduced first-order blood vessels. Both treatment and control CAM had abnormal artery branching and shape. **Conclusion:** According to the findings, *Chicoreus ramosus* contains antioxidant, anti-inflammatory, and anti-tumour angiogenic and proliferative properties and might be a source for the creation of novel anti-cancer drugs.

Keywords: *Chicoreus ramosus*, Antioxidant, Anticancer, Anti-inflamatory, Angiogenic activity.

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INTRODUCTION

Marine organisms provide bioactive molecules. In recent decades, several marine-derived therapeutic agents have entered clinical trials. Molluscs are a source of bioactive compounds. They contain valuable nutrients for all ages. Marine and freshwater products are popular as nutraceuticals, functional foods, and drug and health food ingredients.[1] In ancient Jewish, Christian, and Arabian Muslim faiths, the operculum of certain gastropods was used as incense. In the Middle East, *Strombus tricornis* and *Lambis truncata* sebae operculum are used. Chinese and Japanese incense makers use operculum powder.[2,3] Lung carcinomas originate in either the tracheobronchial mucosa or in the alveolar epithelial cells of the respiratory parenchyma.[4] So far, around 22,000 natural products of marine origin have been identified, with terrestrial sources contributing a larger pool of 131,000 natural products.. Sponge are 37% of coelenterates, and 18% of microorganisms are the primary sources of biomedical compounds, accompanied by other marine invertebrates.[5] Ziconotide, a peptide isolated from a species of tropical marine cone snail, is the first marine-derived medicine. Slugs, clams, mussels, oysters, scallops, squid, octopus, and so on are all molluscs that can be found in marine and estuarine settings.[6] In clinical trials of anticancer drugs, natural chemicals derived from molluscs and their structural counterparts are particularly well represented.[7] The use of certain molluscs in medicinal remedies, such as terrestrial pulmonates, dates back to ancient Rome.[8] It is expected that peptides derived from marine gelatin will have a strong antioxidant effect.[9] In addition to replacing synthetic antioxidants, marine-associated bioactive molecules with anti-oxidative properties could be employed in nutraceuticals and pharmaceuticals.[10] The oxidation process in our bodies causes cellular damage, melanoma, and neurodegenerative disorders; antioxidant molecules found in various molluscs protect cells from oxidation reactions.[11] Free radicals are charged

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molecules with an electronic configuration, which causes them to strive out and encapsulate electrons from other substances to neutralise themselves. However, the harmful effects of free radicals can be mitigated by antioxidant substances that scavenge free radicals and detoxify the organism.[12] A crucial role in combating cardiovascular disease, cancer, neurological illnesses, inflammation, and issues related to cellular and skin aging has been attributed to foods rich in antioxidants.[13] An antioxidant is a molecule that can slow or stop the oxidation of other molecules. Antioxidant molecules found in various molluscs protect cells from oxidation reactions.[11] To assess the antioxidant activity of the gastropods *T. brunneus, C. annulus*, and *B. spirata's* whole body tissue.[3] For centuries, natural products have played a crucial role as primary inspirations for drug discovery efforts.

Currently, 31 marine-derived compounds are progressing through the clinical trials pipeline in pharmaceutical research, and seven have received approval from the Food and Drug Administration (FDA).[14] A significant number of anti-cancer drug leads have been isolated from marine molluscs in particular.[14] Exploring the untapped potential of natural compounds extracted from marine mollusks, including the New Zealand green-lipped mussel *Perna canaliculus*, which has been clinically tested and is now available as a nutraceutical for chronic inflammation treatment, is just the tip of the iceberg.[15] Preliminary studies on whelks, the intriguing predatory gastropods in the *Muricidae* family, have ignited excitement due to their promising secondary metabolites with anti-inflammatory characteristics, as evidenced by crude extract analysis.[16,17] The intricate process of tumorigenesis, hinging on angiogenesis or the formation of new blood vessels in cancer, has become the focal point of recent research efforts.[18] The quest to unravel the precise triggers and inhibitors of this phenomenon has garnered substantial attention.[19] Thus, our study delves into the multifaceted potential of marine gastropods, examining their antioxidant, anti-inflammatory, and anti-angiogenic properties with utmost fascination.

MATERIALS AND METHODS

Assemblage of marine gastropods

The sea gastropod known as *Chicoreus ramosus* was gathered from the shores of Tuticorin, situated along the Gulf of Mannar in Tamil Nadu, India, at coordinates 8.30° N and 7.40° E. These live mollusks were then transferred into a plastic enclosure and subsequently rinsed with tap water to cleanse them.

Preparation of the operculum

Broken shells were employed to extract the operculum from sun-dried soft bodily tissue. Subsequently, a mixer was utilized to grind the dried operculum into a fine powder. The dried powder constituents were then soaked in 100% chloroform for duration of 10 days. Afterward, we filtered the extracts through Whatman No.1 filter paper, concentrating the solvents in a rotary evaporator (VC100A Lark Rotavapor®) at 30°C under reduced pressure to obtain a dark brown sticky mass. To preserve these materials for future research, the residues were frozen.

Anti-proteinase assay

A modification was made to the proteinase inhibitory test procedure.[20] The reaction mixture, consisting of 2 mL, included 0.06 mg of trypsin, 1 mL of Tris-HCl buffer (20 mM, pH 7.4), and various concentrations of *C. ramosus* extract: 500 µg/mL, 250 µg/mL, 100 µg/mL, 50 µg/mL, and 10 µg/mL. The reaction mixture, maintained at 37°C, had 1 mL of 0.8% (w/v) casein added after 5 min, followed by a 20-min incubation period. The reaction was halted using perchloric acid (2%, 70%), and the resulting turbid material was centrifuged. The supernatant's absorbance was measured against Tris-HCl buffer. Each experiment was performed in triplicate, and the entire procedure was repeated three times.

Antioxidant Activity

DPPH Radical Scavenging Activity

To create a 0.1 mM DPPH solution in methanol, 100 mL of the solution is combined with 300 mL of *C. ramosus* extract at various concentrations: 10 µg/mL, 50 µg/mL, 100 µg/mL, 250 µg/mL, and 500 µg/mL, as outlined in reference.[21] These mixtures are allowed to incubate for 30 min at room temperature. Subsequently, the absorbance at 517 nm is measured using a UV-vis spectrophotometer. As a reference, ascorbic acid can be mentioned. Lower absorbance values in the reaction mixture indicate greater free radical scavenging activity, reflecting a higher level of free radical neutralization.

Hydrogen Peroxide Scavenging Activity

H_2O_2 scavenging was tested using *C. ramous* extract.[11] Hydrogen peroxide solution in phosphate buffer (1 M pH 7.4) (43 mM). Various concentrations of *C. ramous* were introduced into 0.6 mL of a 43 mM hydrogen peroxide solution. Hydrogen peroxide absorbance at 230 nm was measured after 10 min against a phosphate buffer blank solution. Ascorbic acid. % inhibition measures free radical scavenging.

Proton Nuclear Magnetic Resonance (^{1}HNMR) analysis

The extract was recorded on an NMR-400 MHz with 1H, 19F, and 15N. We noticed 1H, 19F, and 15N chemical changes. The output graph was compared to the reference chart to assess mushroom function.[22]

Anti-inflammatory activity-Inhibition of albumin denaturation

Inflammation is caused by protein denaturation, hence modest changes were utilized to measure inhibition.[23] *C. ramosus* extract (500 µg/mL, 250 µg/mL, 100 µg/mL, 50 µg/mL, and 10 µg/mL)

combine it with 500 mL of 1% bovine serum albumin. Allow the mixture to sit at room temperature for 10 min and then heat it at 51°C for 20 min. After cooling to room temperature, 660 nm absorbance was measured. Positive control of Acetylsalicylic acid.

Anti-angiogenic activity

The assessment of the anti-angiogenic potential of the test sample extract was conducted using the Chorioallantoic Membrane (CAM) assay, as described in reference.[24] Fertilized chicken eggs were provided by a poultry supplier in Tuticorin, Tamil Nadu, and were incubated for 3-4 days at 37°C in a humidified incubator. After incubation, the embryonic head was identified, and a flashlight was used to mark it in 7-day-old eggs. Subsequently, a small hole was carefully punctured at the narrow end of the eggs using an 18-gauge hypodermic needle, allowing for the withdrawal of 0.5-1.0 mL of albumin, thus separating the yolk sacs from the shell membrane. The embryo's air sac was exposed by removing the shell and membrane using forceps. A 100 g/mL test sample was applied onto a Whatman No.1 filter paper disc, which was then placed on the CAM and incubated for 8 days. After 3 days of incubation, when the CAM was dissected from the eggs, the number of blood vessels, particularly centrally converging vessels, was quantified using a microscope. Each sample was evaluated using twenty or more eggs.

Cell culture and MTT assay

In each well of a 96-well plate, 1104 MG-63 bone osteosarcoma cancer cells were individually seeded and then placed in a CO_2 incubator at 37°C with 5% CO_2. The cells were cultured in MEM media supplemented with 1X Antibiotic Antimycotic Solution and 10% fetal bovine serum (Himedia, India). Following a 24-hr incubation period in serum-free media, the cells were washed twice with 200 µL of 1X PBS. Subsequently, following the cell treatment, the culture medium was removed by aspiration. The MTT assay was conducted by incubating the cells with 0.5 mg/mL of MTT in 1X PBS at 37°C inside a CO_2 incubator for 4 hr. After incubation, the cells were washed twice with 200 µL of MTT-containing PBS. The crystals were dissolved using 100 µL of DMSO, resulting in a blue-violet coloration. The absorbance at 570 nm was measured using a microplate reader, as outlined in reference.[25]

Statistical analysis

The mean standard deviation represents values. Analysis of Variance (one-way and two-way) was used to compare experimental group means with normal groups. All statistical analyses were done in SPSS.

RESULTS AND DISCUSSION

Hydrogen peroxide scavenging assay

Figure 1 illustrates the concentration-dependent scavenging effect of the chloroform extract from *C. ramosus* operculum on hydrogen peroxide (H_2O_2) radicals at various concentrations (10 µg/mL, 50 µg/mL, 100 µg/mL, 250 µg/mL, and 500 µg/mL). This extract significantly inhibited oxidative stress induced by H_2O_2. In comparison to the control group, the observed differences are statistically significant at a *p*-value of less than 0.05. With increasing extract concentration, the percentage inhibition showed an ascending trend, measuring 32.31%, 35.81%, 41.89%,

Table 1: DPPH radical Scavenging activity of *C. ramosus* operculum extract

Concentration (µg/mL)	OD Value (517 nm)	% Inhibition
Control	1.870±0.031	-
500 µg/mL	1.122±0.078	39.98
250 µg/mL	1.261±0.015	32.53
100 µg/mL	1.293±0.023	30.83
50 µg/mL	1.33±0.009	28.75
10 µg/mL	1.525±0.037	18.41
Ascorbic acid	0.103±0.020	94.47

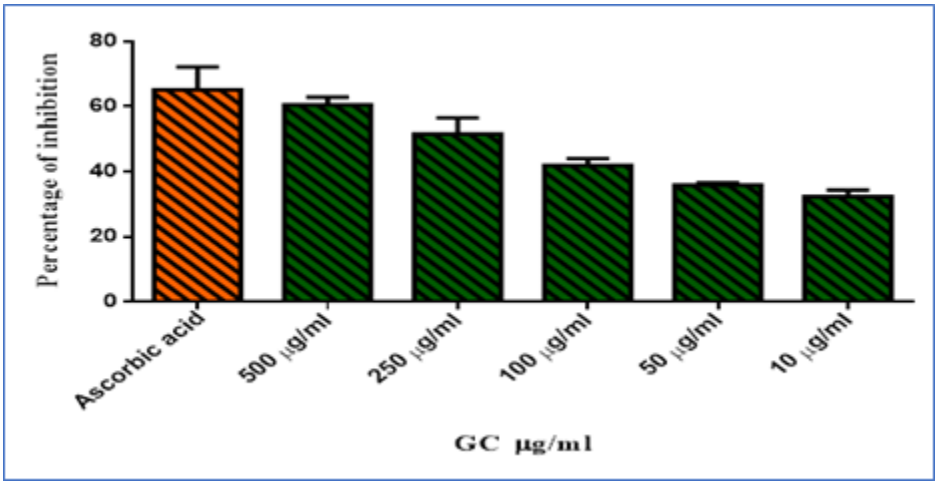


Figure 1: H_2O_2 scavenging activity of chloroform extract of *C. ramosus* operculum.

51.57%, and 60.47% for the respective concentrations, while ascorbic acid exhibited a 65.09% inhibition rate. The highest scavenging capacity was observed at the concentration of 500 µg/mL, indicating a direct relationship between concentration and scavenging activity.

2,2-Diphenylpicrylhydrazyl (DPPH): Radical Scavenging Activity

The different concentration of *C. ramosus* operculum chloroform extract (10 µg/mL, 50 µg/mL, 100 µg/mL, 250 µg/mL, and 500 µg/mL) were shown to have effects on oxidative damage generated by hydroxyl radical ranging from 18.41% to 94.47%. Maximum inhibition was seen at a concentration of 500 µg/mL with a threshold of 10 µg/mL. Antioxidants reduce DPPH absorbance by causing a chain reaction between antioxidant molecules and radicals, which ultimately leads to hydroxyl radical scavenging Table 1.

Protease inhibitory assay

The study investigated the impact of various concentrations of chloroform extract from *C. ramosus* operculum and naturally occurring flavonoid compounds. *C. ramosus* demonstrated pronounced inhibitory effects on proteases in rats. When compared to the lowest concentration of 10 µg/mL, the *C. ramosus* operculum extract at 500 µg/mL resulted in a significant 58.98% reduction in protease activity ($p<0.05$), as illustrated in Figure 2.

Table 2: **Inhibition of albumin denaturation activity of *C. ramosus* operculum extract**

Concentration (µg/mL)	OD Value (517 nm)	% Inhibition
Control	2.168±0.004	-
500 µg/mL	1.061±0.058	39.98
250 µg/mL	1.249±0.023	32.53
100 µg/mL	1.337±0.008	30.83
50 µg/mL	1.402±0.036	28.75
10 µg/mL	1.741±0.005	18.41

Anti-Inflammatory activity: Inhibition of albumin denaturation

The anti-inflammatory properties of flavonoids extracted from *C. ramosus* operculum were evaluated based on their impact on albumin denaturation. The extract, at various concentrations, exhibited a significant inhibition of albumin denaturation when compared to the control group ($p<0.05$). Similar inhibitory effects were observed with diclofenac sodium. Notably, the extract's inhibition showed a concentration-dependent pattern, with 10 µg/mL resulting in an 18.41% inhibition and the highest concentration of 500 µg/mL demonstrating the most substantial inhibition at 39.98%. Furthermore, the total flavonoids exhibited a significant and dose-dependent capacity to inhibit the increase in paw edema, as indicated in Table 2.

Anti-angiogenic activity

The flavonoids extracted from *C. elegans* have been found to possess anti-inflammatory properties.[32] In the context of *C. ramosus* operculum's impact on albumin denaturation, it was observed that all concentrations of the extract significantly ($p<0.05$) prevented albumin denaturation, mirroring the pattern observed with diclofenac sodium. The extract's inhibitory effect increased with rising concentrations, reaching a maximum inhibition of 39.98% at 500 µg/mL and a minimum inhibition of 10.41% at 10 µg/mL. Furthermore, the total flavonoids demonstrated a dose-dependent slowing of paw edema development, as shown in Table 2.

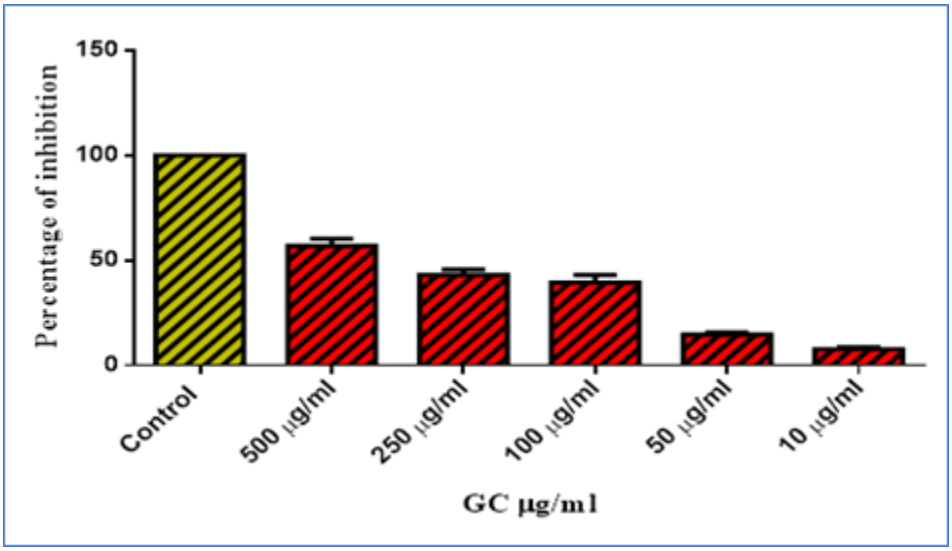


Figure 2: Protease inhibitory Activity of chloroform extract of *C. ramosus* operculum.

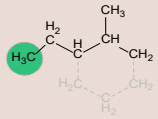
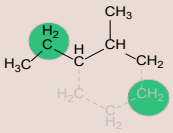
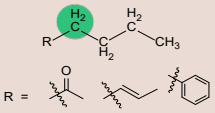
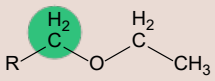
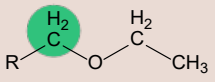
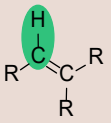
Angiogenesis in chick embryo

In Plate 1, a dose-dependent reduction in the number of branching points and first-order blood vessels was observed. A notable 44% reduction in first-order blood vessels ($p<0.001$) and a reduction in branching points were seen at a concentration of 100 µg/mL. Analysis of scanned CAM cases, both treated and untreated, revealed distinct deviations in the pattern and morphology of blood vessel branching. In blind tests, untreated areas were easily distinguishable from treated ones. Additionally, as depicted in Plate 1, treated CAMs exhibited anomalies in blood vessel patterns, including disorganization of branching and an increased occurrence of parallel blood vessels without branching. Furthermore, the treated CAMs displayed thinner and fewer blood vessels compared to the controls. Both the average Vascular Diameter (DV) and Total Length (LT) showed consistent trends in terms of percent obstruction, indicating anti-vascular effects of the crude extract Figure 4.

HNMR identification of compounds in *C. ramosus* operculum extract

Table 3 and Figure 5 present the *C. ramosus* operculum powder along with characteristic signals corresponding to identify metabolites. The results revealed distinct spectral patterns, including high-intensity peaks of aliphatic compounds with methyl protons in the range of 0.877 ppm to 0.841 ppm. Additionally, low-intensity peaks of aliphatic/alicyclic compounds with methylene protons at 1.250 ppm and high-intensity peaks of methylene protons adjacent to alkene, carbonyl, or aromatic rings at 2.332 ppm were clearly observable. Signals corresponding to alkyl chains attached to oxygen appeared at 4.079 ppm and 4.326 ppm, while those associated with alkenes were evident at 5.780 ppm. This extract not only supports biological research but also holds promise for the development of medicinal resources. Six distinct carbon signals were evident in the HNMR spectrum, excluding the glycone carbons. These signals appeared at chemical shifts of 102.2, 73.5, 74.0, 71.8, 76.5, and 171.3 ppm, indicating the presence of a β-D-glucuronyl group. This was further confirmed

Table 3: NMR (CDC13) spectral data for compounds of *C. ramosus* operculum extract

Peak	Expected groups	Chemical structure
High intensity peaks		
0.877-0.841	Aliphatic compounds with Methyl protons	
1.250	Aliphatic/Alicyclic compounds, with Methylene protons	
Low intensity peaks		
2.332	Methylene protons adjacent to, alkene or carbonyl or aromatic ring	
4.079	Alkyl chain attached to Oxygen	
4.326	Alkyl chain attached to Oxygen	
5.780	Alkene	

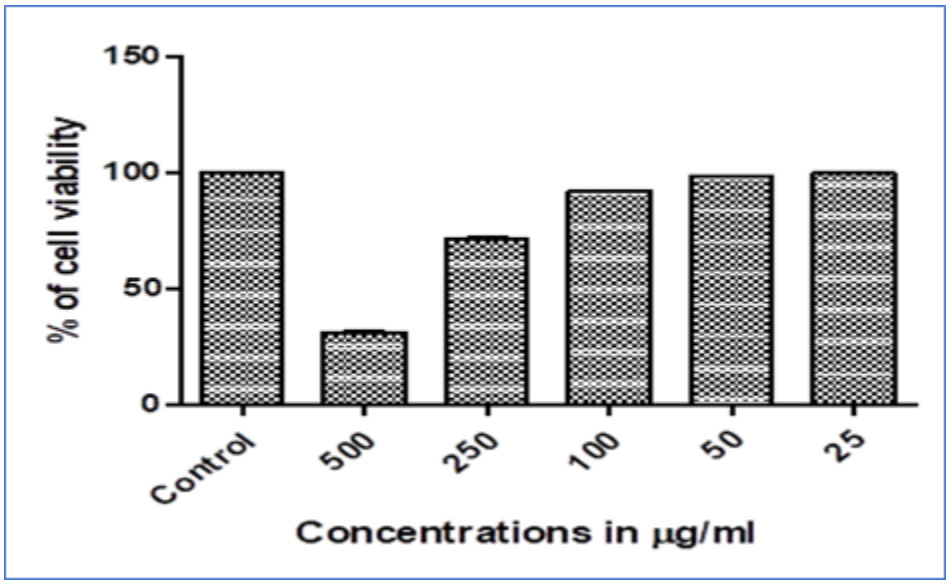


Figure 3: MTT and cell viability activity of *C. ramosus* operculum extract.

by the coupling constant of the anomeric protons, observed at 5.09 ppm.

MTT and cell viability assay

The cytotoxicity of the *C. ramosus* operculum chloroform extract against the Bone Osteosarcoma cancer cell line (MG-63) increased with increasing concentrations (Figure 3). In a 1:4 dilution, the experiment showed an IC_{50} (half maximum inhibitory concentration) of roughly 100 µg/mL. Variable doses of *C. ramosus* operculum extract on the viability of MG-63 cells. Comparing extract concentrations of 500 µg/mL and 10 µg/mL, MG-63 cell viability varied from 31.33% to 99.73%. MG-63 cell viability and morphology under the microscope. Plate 2 explains the microscopic images.

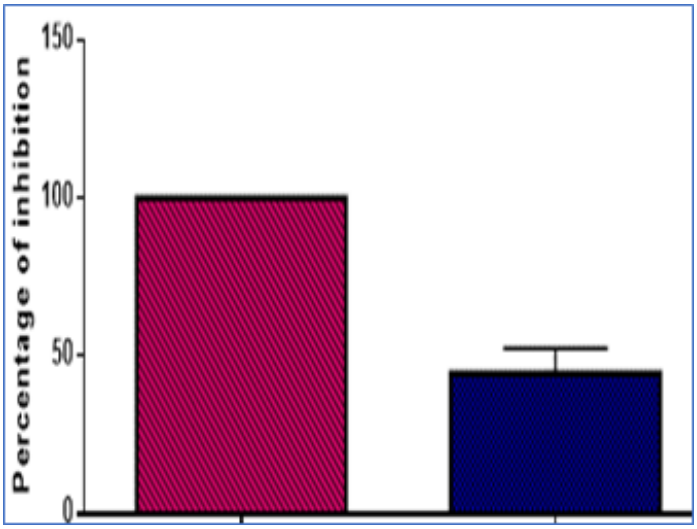


Figure 4: Effect of GC sample discs on angiogenesis in chick embryo CAM assay of *C. ramosus* operculum extract.

DISCUSSION

Bijayalakshmi Devi Nongmaithem *et al.*, 2017 reported Marine *Gastropod operculum* has gained attention for its potential pharmacological properties, especially due to the presence of bioactive compounds that may exhibit antimicrobial, anti-inflammatory, and analgesic effects. Hydrogen peroxide scavenging assay of Marine molluscs extract of *M. meretrix* and *M. casta* showed potential antioxidant, radical scavenging, reducing power and metal chelating abilities Sugesh *et al.*, 2019. Jenifer and Brisca Renuga, 2024 findings confirmed that the marine gastropod of *P. sulcatus* chloroform extracts contain a diverse range of bioactive compounds which could contribute as a valuable resource for realizing novel therapeutic compounds, developing innovative treatments, and contributing to pharmaceutical and biomedical research. Notably, H_2O_2 is known for its ability to penetrate biological membranes, and while it is not highly reactive itself, its hydroxyl radicals can be extremely damaging to cells.[26] In contrast to being a strong scavenger of hydrogen peroxide, the scavenging activity of protein at various concentrations was observed, with the maximum activity observed in *P. virens* methanolic hydrogen peroxide free radicals.[27] In this study, the methanolic extract of *C. ramosus* at concentrations ranging from 20 µg/mL to 250 µg/mL displayed scavenging percentages of 67.09%, 74.83%, 60.21%, and 59.58%, respectively. These findings suggest that the *C. ramosus* crude extract may possess significant potential in scavenging H_2O_2, potentially attributed to the high concentration of proteins and free radicals present in the extract. Overall, the results highlight the concentration-dependent H_2O_2 scavenging activity of the extract, possibly due to the presence of antioxidants, and its noteworthy inhibitory effect on oxidative stress. Antioxidants

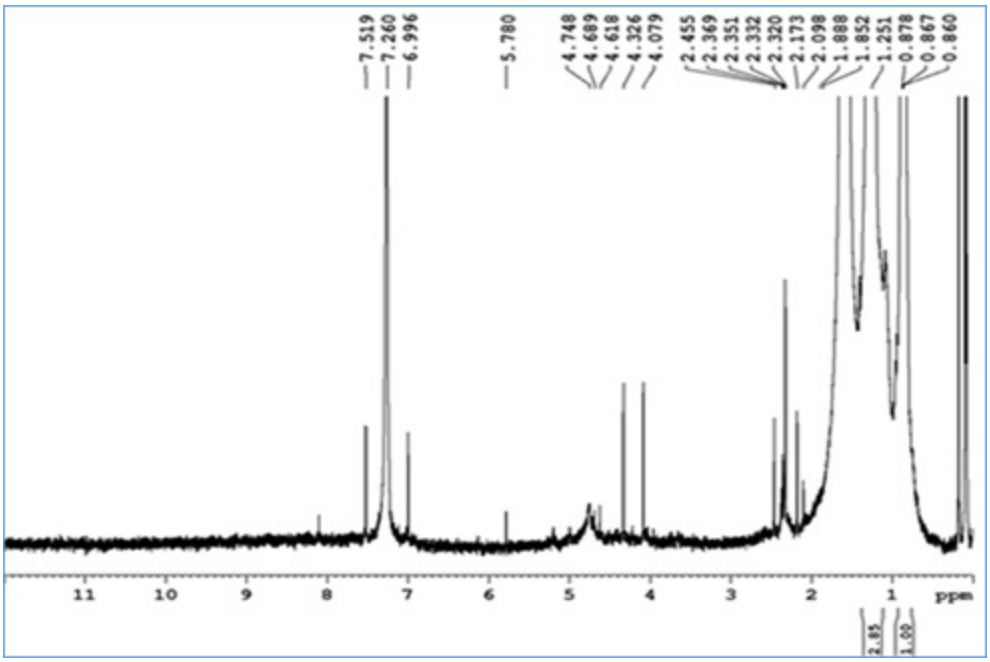
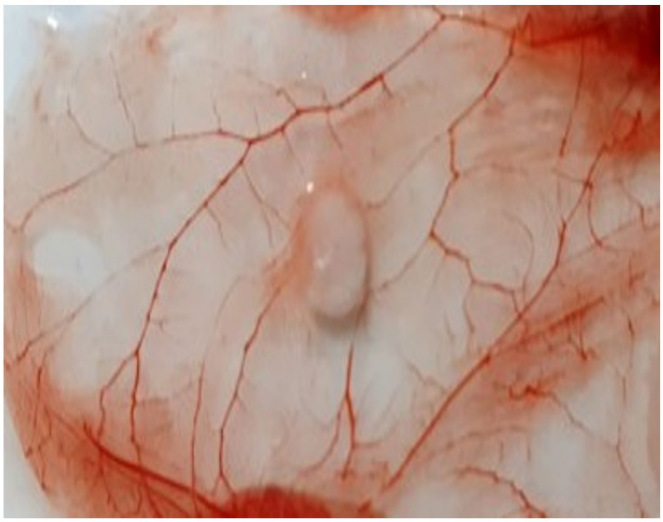
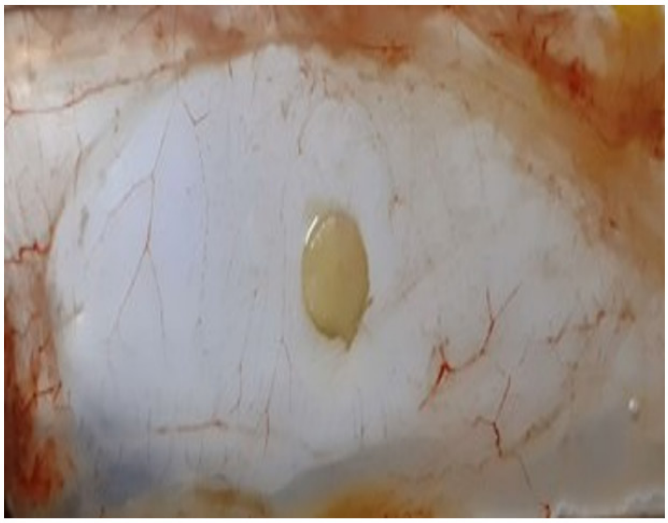


Figure 5: NMR data of *C. ramosus* operculum extract.



Control



Treated with 100 µg/mL.

Plate 1: Effect of angiogenesis in chick embryo CAM assay of *C. ramosus* operculum extract.

and radical scavengers can be evaluated quickly, easily, and accurately with the help of the DPPH radical.[28] In order to measure the ability of diverse natural products and some pure manufactured chemicals to scavenge free radicals, the DPPH test is commonly utilized. When the absorbance of the DPPH radical is measured at 517 nm, the colour shift from purple to yellow due to the presence of antioxidants is readily apparent.[29] The extracts' antioxidant capacity can be gauged by how much colour they remove. The chloroform extracts of *C. ramosus* operculum demonstrated possible antioxidant activity in the present study.

Protease inhibitors are indispensable tools for elucidating the fundamental principles of protein interactions. Enzymes responsible for protein degradation, such as bromelain, papain, pancreatin, trypsin, chymotrypsin, and rutin, play crucial roles in regulating and modulating inflammatory responses. Reports indicate that during inflammatory reactions, leukocyte proteases play a crucial role in causing tissue damage, and significant protection can be provided using protease inhibitors.[30] Neutrophils, which contain serine proteases, are localized in lysosomes and are recognized as a rich source of these enzymes.[29] The highest dosage of total flavonoids displayed the most potent anti-inflammatory effects. It's worth noting that marine invertebrates, thriving in diverse environments, serve as valuable sources of a wide range of pharmacologically active substances.[31,32] Among these invertebrates, molluscs have been identified as possessing bioactive compounds of biomedical importance and have evolved highly effective innate immune mechanisms.[33] The most robust anti-inflammatory effects were observed at the highest total flavonoid dose. It is noteworthy that molluscs are one of the invertebrate groups known to produce biomedically significant compounds and have evolved highly effective innate immune mechanisms.[33] Marine invertebrates, thriving in diverse environments, serve as rich sources of a wide range of pharmacologically active substances.[34,35] NMR studies of whole tissue and specific tissues of marine molluscs revealed the presence of other free amino acids and sugars, confirming the presence of taurine, betaine, and glycine in the body.[36] The anti-cancer effect of the extract was confirmed by a dose-dependent reduction in MG-63 cell viability, with an IC_{50} value of around 100 µg/mL. Several plant-based compounds have been described by other writers to exhibit promising anticancer effects against MG-63 cells. When compared to the IC_{50} values reported for other plant extracts.[37] and previous research, it appears that the methanolic extract of *C. quadrangularis* has a strong inhibitory effect on MG-63 cells. Comparison of the anti-oxidant and anticancer activity of chloroform and ethanol extracts of *Cissus quadrangularis* leaves was performed in an *in vitro* investigation. Results showed that ethanol extract was superior to chloroform extract in terms of both qualities. The MTT assay and the tryptan blue method both showed that a chloroform extract of *C. ramosus* operculum had strong anticancer activity against Ehrlich Adenocarcinoma cell lines.

The chloroform extract of *C. ramosus* operculum showed cytotoxicity against the Bone Osteosarcoma cancer cell line (MG-63) in a concentration-dependent manner (Figure 3). IC_{50} revealed by the assay was around 100 µg/mL at a dilution of 1:4. Feasibility of MG-63 cells with various concentrations of *C. ramosus* operculum extract. The cell viability of MG-63 cells ranged between 31.33% and 99.73% at extract concentrations of 500 µg/mL and 10 µg/mL, correspondingly. Cell viability and cytological characteristics of MG-63 cells. The microscopic images are described in Plate 2. As the dose of the extract

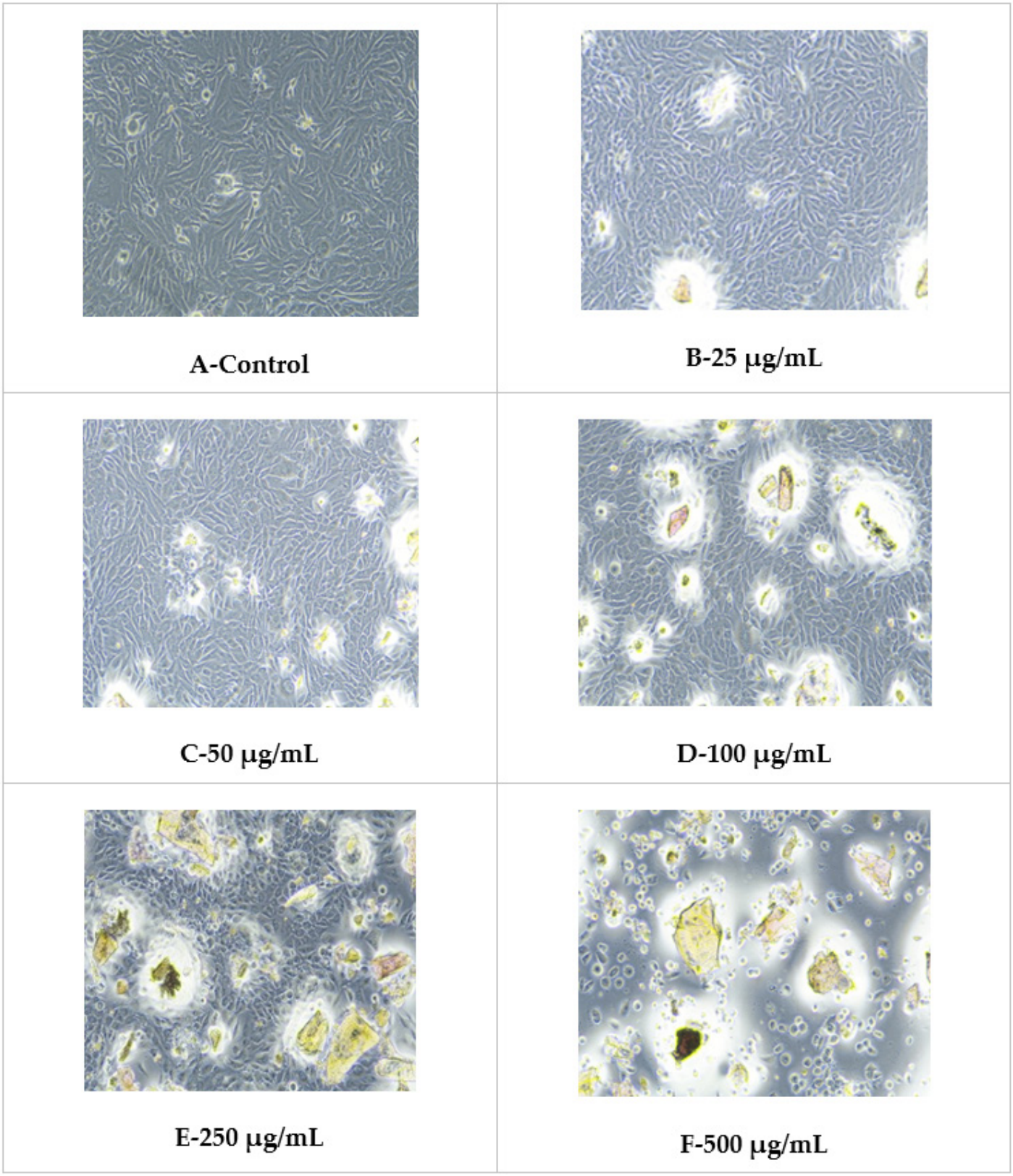


Plate 2: Anticancer activity of *C. ramosus* operculum extract on MG63 osteosarcoma cells. A-Control cells, B-Cell viability at 25 µg/mL, C-Cell viability at 50 µg/mL, D-Cell viability at 100 µg/mL. E-Cell viability at 250 µg/mL, F-Cell viability at 500 µg/mL.

increased, the cell viability of MG-63 cells decreased, affirming the anti-cancer properties of the extract with an observed IC_{50} value of approximately 100 g/mL. Previous studies by other authors have reported various naturally derived plant products showing potential anticancer effects against MG-63 cells. A comparison of the IC_{50} values from these studies indicates that the methanolic extract of C. quadrangularis demonstrates substantial inhibition of MG-63 cells.[38-40] During an *in vitro* study, the antioxidant and anticancer activities of leaf extracts from. *Cissus quadrangularis* were examined, with comparisons between chloroform and ethanol extracts. The results revealed that the ethanol extract exhibited superior properties in both antioxidant and anticancer activities compared to the chloroform extract. The chloroform extract of *C. ramosus* operculum showed potent anticancer activity against Ehrlich Adenocarcinoma cell lines, which was demonstrated by the MTT assay as well as the tryptan blue method.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ABBREVIATIONS

GC: Writing – original draft, Writing – review and editing. **PI:** Software, Supervision, Validation. **CM and PP:** performance experimental work.

SUMMARY

Our research has led to the discovery of a potent anti-inflammatory and antiangiogenic protein within the *C. ramosus* operculum extract. This extract demonstrated impressive antioxidant properties and effectively neutralized hydrogen peroxide. These beneficial effects can be largely attributed to its abundant flavonoid content. These findings indicate the extract's potential to hinder tumor growth by inhibiting both blood vessel formation and cell division. This study underscores the valuable reservoir of antioxidant compounds found in marine species, offering promising prospects for future developments in pharmacology, industry, and biotechnology products.

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