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Research Article

Anticancer Activity and Relative Gene Expression of βGBP in the Serum of Mole Crab, *Emerita asiatica*

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ABSTRACT

Background: β-1,3 Glucan Binding Protein (βGBP) is an important immune-related protein in crustaceans with potential biomedical applications. The present study evaluated the gene expression, anticancer, and antimicrobial activities of βGBP isolated from the serum of *Emerita asiatica*. **Materials and Methods:** βGBP was isolated using affinity chromatography and analyzed through nested PCR and qPCR. Antiproliferative activity was evaluated against HepG2, EAC, MCF-7, A375, HeLa, and L929 cell lines using MTT assay. Apoptotic activity and p53 expression were assessed by AO/EB staining and flow cytometry. Antibacterial and antifungal activities were determined by disc diffusion assay. **Results:** qPCR analysis confirmed differential expression of βGBP genes in *E. asiatica*. Purified βGBP exhibited significant antiproliferative activity, particularly against HepG2 and EAC cell lines. AO/EB staining and flow cytometry demonstrated apoptosis induction and enhanced p53 expression in HepG2 cells. βGBP also showed broad-spectrum antibacterial and antifungal activities against tested pathogens. **Conclusion:** The findings demonstrate that βGBP from *Emerita asiatica* possesses significant anticancer and antimicrobial properties, indicating its potential as a promising marine-derived therapeutic biomolecule.

Keywords: Anti-Cancer Activity (ATC), Antimicrobial activity, Apoptosis, β-1,3 Glucan Binding Protein (βGBP), *Emerita asiatica*, Gene expression.

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INTRODUCTION

Cancer remains one of the leading causes of mortality worldwide and continues to pose a major challenge in therapeutic research. Natural products derived from marine organisms have gained increasing attention due to their diverse bioactive compounds and potential pharmacological applications. Marine invertebrates, particularly crustaceans, possess highly efficient innate immune systems that protect them against microbial infections, environmental stress, and pathogenic invasions. Among the important immune molecules identified in crustaceans, β-1,3 Glucan Binding Protein (βGBP) plays a vital role in pathogen recognition, immune defense, phagocytosis, and antimicrobial activity.

In invertebrates, βGBP functions as a pattern recognition receptor involved in the detection of microbial polysaccharides and activation of immune responses. Previous studies have demonstrated that βGBP can agglutinate bacteria, enhance phagocytosis by hemocytes, and participate in the clearance of invading microorganisms from hemolymph (Arimoto and Tripp, 1977; Renwrantz and Stahmer, 1983). Crustacean hemolymph contains several immune-related proteins associated with prophenoloxidase activation, encapsulation, melanization, and nonspecific immune defense mechanisms. Despite these observations, the exact contribution of βGBP in crustacean immunity and its biomedical significance remain inadequately explored.

Marine-derived compounds have emerged as promising candidates in anticancer drug discovery. Several clinically important anticancer agents have originated from natural sources, emphasizing the therapeutic importance of marine biomolecules. β-glucans and their associated proteins have been reported to exhibit immunomodulatory, antimicrobial, antioxidant, and antiproliferative activities. Recent studies demonstrated that βGBP and related compounds could suppress tumor proliferation, regulate apoptotic pathways, and enhance immune-mediated anticancer responses (Song *et al.*, 2017; Shang *et al.*, 2018). Furthermore, βGBP-coated nanoparticles derived from crustacean serum have shown selective toxicity against HepG2 liver cancer cells and bacterial pathogens (Iswarya *et al.*,2017). These findings indicate the potential therapeutic significance of crustacean-derived βGBP in cancer research.

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Emerita asiatica, commonly known as the mole crab, is a marine crustacean belonging to the family Hippidae and is abundantly distributed along intertidal coastal regions. Due to its adaptation to dynamic shoreline environments and efficient immune responses, this species has attracted attention in biochemical and biomedical investigations. Although several studies have reported the antimicrobial and immunological properties of crustacean serum proteins, limited information is available regarding the anticancer potential and relative gene expression of βGBP isolated from *E. asiatica*.

Therefore, the present study aimed to isolate and characterize β-1,3 glucan binding protein from the serum of *Emerita asiatica* and evaluate its relative gene expression, antiproliferative activity, apoptotic induction, antibacterial, and antifungal properties. The study further investigated the effect of βGBP on different cancer cell lines, particularly HepG2 cells, to determine its therapeutic potential as a marine-derived anticancer biomolecule.

MATERIALS AND METHODS

Sample Collection and Serum Preparation

Healthy specimens of *Emerita asiatica* were collected from the intertidal coastal regions of Chennai, Tamil Nadu, India. The collected mole crabs were washed thoroughly with sterile seawater to remove adhering debris and contaminants. Hemolymph was aseptically collected from healthy individuals and allowed to clot under chilled conditions for serum separation. The serum samples were centrifuged and stored at 4°C until further analysis.

DNA Extraction

Genomic DNA was isolated from the serum samples using DNA extraction buffer. Briefly, 100 µL of serum sample was mixed with 600 µL of extraction buffer and incubated at room temperature for 10 min. The mixture was centrifuged at 500 rpm for 5 min, and the supernatant was transferred to a fresh tube. Two volumes of absolute ethanol were added for DNA precipitation, followed by centrifugation at 10,000 rpm for 10 min. The DNA pellet obtained was washed, air dried, and dissolved in sterile nuclease-free water for subsequent PCR analysis.

PCR and Nested PCR Analysis

PCR amplification was performed using specific primers targeting β-1,3 glucan binding protein (βGBP) genes. The reaction mixture consisted of PCR master mix, forward and reverse primers, and DNA template. Amplification was carried out under optimized thermal cycling conditions using a thermal cycler.

Nested PCR was subsequently performed using Dr. Shahul's nested PCR kit to confirm the presence of βGBP genes in the serum samples. The nested PCR reaction mixture contained PCR master mix, nested primers, and amplified PCR products as template DNA. The amplified products were analyzed using 1% agarose gel electrophoresis and visualized under UV transillumination.

Agarose Gel Electrophoresis

PCR amplified products were analyzed using 1% agarose gel prepared in 1X TAE buffer containing ethidium bromide. The amplified DNA samples and molecular weight marker were loaded into the wells and electrophoresed under standard conditions. The gels were visualized using a UV transilluminator and photographed for documentation.

Hemocyte Separation

Hemocytes were separated according to the method of Smith and Söderhäll (1983). Ice-cold anticoagulant solution containing citrate-EDTA, trisodium citrate, citric acid, NaCl, glucose, and EDTA disodium salt was mixed with the serum samples and centrifuged at 200 × g for 2 min at 4°C. The hemocyte pellet obtained was suspended in iso-osmotic buffer for further studies.

RNA Isolation and cDNA Synthesis

Total RNA was isolated from serum samples using RNA Isoplus reagent. The homogenized samples were centrifuged, and chloroform was added for phase separation. RNA was precipitated using ethanol, washed, air dried, and dissolved in sterile water. The isolated RNA was quantified and used for first-strand cDNA synthesis using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) according to the manufacturer's instructions.

Quantitative PCR Analysis

Relative gene expression of βGBP genes I, II, and III was analyzed by quantitative PCR (qPCR). The reaction mixture consisted of SYBR Green master mix, forward and reverse primers, cDNA template, and nuclease-free water. The 18S rRNA gene was used as the endogenous control. Amplification and melt curve analyses were performed using a real-time PCR system, and relative expression levels were calculated using threshold Cycle (Ct) values.

Isolation and Purification of βGBP

β-1,3 glucan binding protein was isolated from serum using affinity chromatography with β-1,3 glucan-linked agarose. The purified fractions were lyophilized and dissolved in Phosphate-Buffered Saline (PBS) at a concentration of 1 mg/mL prior to biological assays.

Cell Culture

Human cancer cell lines including HepG2 (hepatocellular carcinoma), MCF-7 (breast cancer), HeLa (cervical cancer), A375 (skin carcinoma), Ehrlich Ascites Carcinoma (EAC), and L929 normal fibroblast cells were procured from NCCS, Pune, India. The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum

and incubated at 37°C in a humidified atmosphere containing 5% CO_2.

MTT Assay for Antiproliferative Activity

The antiproliferative activity of purified βGBP was evaluated using MTT assay. Cancer cells were seeded in culture plates and treated with different concentrations of βGBP (6.25-100 µg/mL) for 24 hr. Following incubation, MTT reagent was added and incubated for 3 hr. Formazan crystals formed were dissolved in DMSO, and absorbance was measured at 540 nm using a microplate reader. The IC_{50} values were calculated based on percentage cell viability.

AO/EB Dual Staining Assay

Apoptotic cell death was analyzed using Acridine Orange and Ethidium Bromide (AO/EB) dual staining method. HepG2 cells treated with βGBP were washed with PBS and stained with AO/EB solution. The stained cells were observed under a fluorescence microscope to identify morphological changes associated with apoptosis.

Flow Cytometry Analysis of p53 Expression

The expression of p53 protein was evaluated by flow cytometry. βGBP-treated cells were trypsinized, washed with PBS, and fixed using Cytofix/Cytoperm solution. Anti-p53-PE antibody was added and incubated in the dark for 30 min. The samples were analyzed using flow cytometry, and mean fluorescence intensity was recorded using Cell Quest Pro software.

Antibacterial Assay

The antibacterial activity of βGBP was assessed using the disc diffusion method against bacterial pathogens including *Escherichia coli, Staphylococcus aureus, Salmonella paratyphi, Pseudomonas aeruginosa, Bacillus subtilis*, and *Streptococcus pyogenes*. Sterile discs impregnated with different concentrations of βGBP were placed on Mueller-Hinton agar plates inoculated with test organisms. Streptomycin served as the positive control. After incubation at 37°C for 24 hr, the zones of inhibition were measured.

Antifungal Assay

Antifungal activity was evaluated by the spread plate technique against fungal pathogens including *Candida albicans, Aspergillus niger, Mucor* sp., *Rhizopus* sp., *Aspergillus flavus*, and *Aspergillus fumigatus*. Discs containing purified βGBP were placed on inoculated plates, and ketoconazole was used as the positive control. The plates were incubated at 37°C for 2-4 days, and antifungal activity was determined by measuring inhibition zones.

Amino Acid Composition Analysis

The amino acid composition of purified βGBP was analyzed using High-Performance Liquid Chromatography (HPLC). The occurrence frequency of essential and non-essential amino acids was determined and compared with standard amino acid profiles to evaluate the biochemical properties of the purified protein.

Statistical Analysis

All experiments were carried out in triplicate, and the results were expressed as Mean±Standard Deviation (SD). Statistical analysis was performed using appropriate statistical software. Differences among groups were analyzed using one-way ANOVA, and p-values <0.05 were considered statistically significant.

Ethical Statement

All experimental procedures involving marine organisms and cell lines were conducted in accordance with institutional ethical guidelines.

RESULTS

Detection and Relative Gene Expression of βGBP in *Emerita asiatica*

Nested PCR analysis confirmed the presence of β-1,3 Glucan Binding Protein (βGBP) genes in the serum samples of *Emerita asiatica*. Agarose gel electrophoresis revealed distinct amplified bands corresponding to the expected amplicon size of approximately 310 bp in the test samples and positive control, while no amplification was observed in the negative control. Total RNA isolated from serum samples exhibited good integrity and was successfully converted into cDNA for further gene expression studies.

Quantitative PCR analysis demonstrated differential expression of βGBP genes I, II, and III in the serum samples. Among the analyzed genes, βGBP I exhibited comparatively higher relative expression levels than βGBP II and III. Melt curve analysis confirmed the specificity of amplification without significant nonspecific products. These findings suggest the active involvement of βGBP genes in the immune response mechanisms of *E. asiatica*. The molecular detection of βGBP genes through DNA extraction, PCR amplification, nested PCR, RNA isolation, and cDNA analysis is presented in Figures 1a-1e. The melt curve and relative expression analyses of βGBP I, II, and III are illustrated in Figures 2a-2f. The primer sequences used for amplification and qPCR analysis are listed in Table 1, while the relative gene expression profile of βGBP genes is summarized in Table 2.

Antiproliferative Activity of βGBP

The antiproliferative activity of purified βGBP was evaluated against different human cancer cell lines including HepG2, EAC, MCF-7, A375, and HeLa cells using MTT assay. βGBP demonstrated significant dose-dependent cytotoxicity against all tested cancer cell lines. Among them, HepG2 cells exhibited the highest sensitivity with an IC_{50} value of 40.84 µg/mL, followed

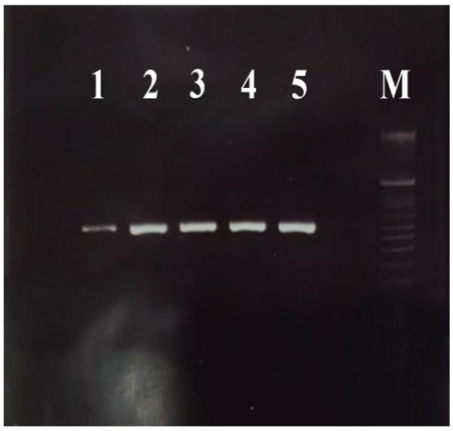
Table 1: Primer sequences used for amplification and qPCR analysis of βGBP genes.

Sl. No.	Gene	Sequence	Reference
1.	βGBP IF	5′AGTGAAGCCATTGAAGCAGCG3′	(Zhao *et al.*, 2017)
2.	βGBP IR	5′ GGTCCGGATGACGACTGTAACTG 3′	(Zhao *et al.*, 2017)
3.	βGBP IIF	5′ TTGAGGCTGCTGCCGTCCA 3′	(Zhao *et al.*, 2017)
4.	βGBP IIR	5′ TCCGGGTCAAAGGAATCAGC 3′	(Zhao *et al.*, 2017)
5.	βGBP III F	5′ CAGCTTATCGAGCCAAACAGACG 3′	(Zhao *et al.*, 2017)
6.	βGBP III R	5′CGAAATAGGCAACTCTTTGTCCG 3′	(Zhao *et al.*, 2017)
7.	18SrRNAF	5′ GTAACCCGTTGAACCCCATT 3′	Eurofins
8.	18SrRNAR	5′ CCATCCAATCGGTAGTAGTAGCG 3′	Eurofins

Table 2: Relative gene expression profile of βGBP genes in *Emerita asiatica* serum.

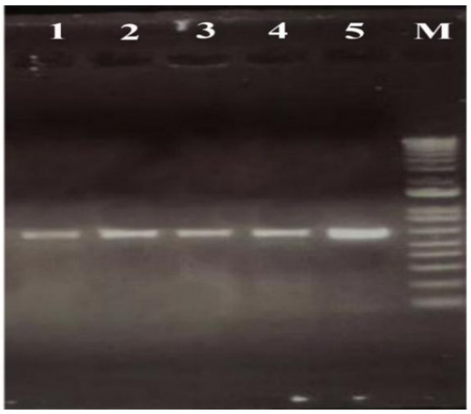
Target Gene	Mean Ct Value	Standard Deviation (Mean)	Relative Expression Level
18S rRNA	24.29	2.63	Endogenous control
βGBP I	26.77	2.71	High
βGBP II	26.31	0.85	Moderate
βGBP III	29.69	0.36	Low

1a: DNA Extraction



1b: First PCR Amplification

1c: Nested PCR Amplification



1d: Total RNS isolation

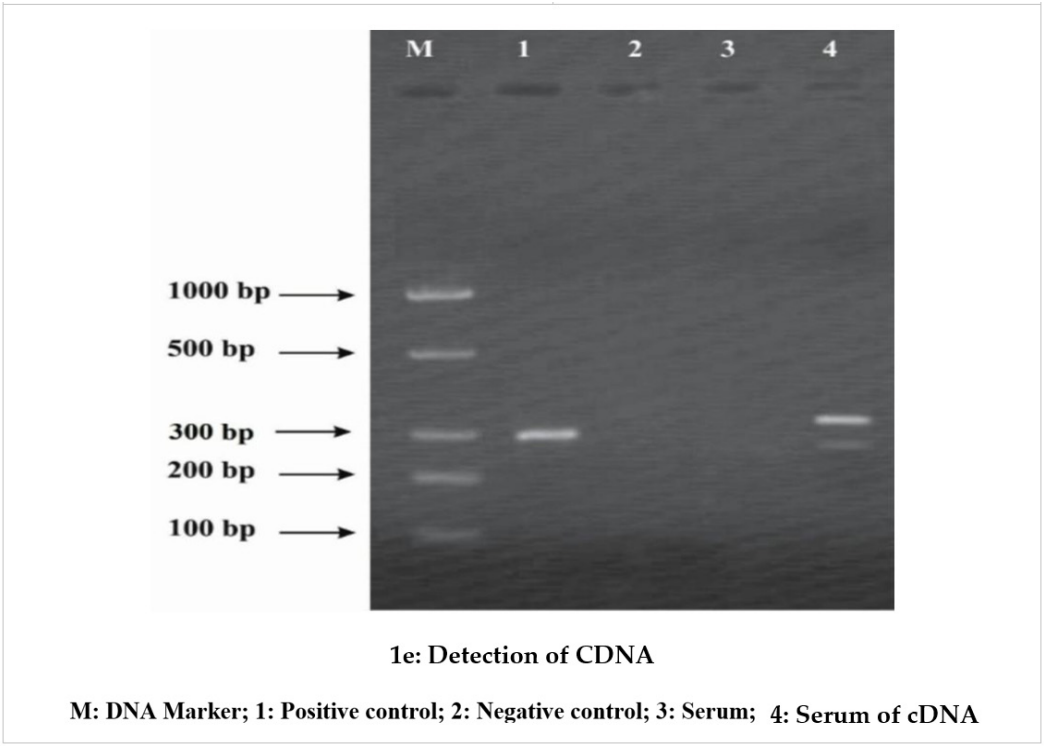


Figure 1a-1e: Molecular detection and expression analysis of βGBP genes in *Emerita asiatica*.

by EAC cells with an IC_{50} value of 44.75 µg/mL. In contrast, the normal fibroblast cell line L929 showed comparatively higher resistance, indicating selective anticancer activity of βGBP.

Morphological observations revealed membrane shrinkage, irregular cell morphology, rounding of cells, and cellular detachment in treated HepG2 cells. The degree of morphological alteration increased with increasing concentrations of βGBP, confirming its antiproliferative effect. The IC_{50} values and dose-dependent antiproliferative activity of βGBP against different cancer cell lines are presented in Figure 3 and Table 3, respectively.

Apoptotic Activity and p53 Expression Analysis

AO/EB dual staining analysis demonstrated apoptotic cell death in βGBP-treated HepG2 cells. Untreated cells exhibited normal green fluorescence, whereas treated cells showed orange-red fluorescence with chromatin condensation and nuclear fragmentation, indicating late-stage apoptosis. The apoptotic effect increased progressively with increasing concentrations of βGBP. Flow cytometry analysis further confirmed apoptosis induction through enhanced p53 expression in βGBP-treated HepG2 cells. The treated cells exhibited significantly elevated mean fluorescence intensity compared to untreated controls, suggesting activation of p53-mediated apoptotic pathways**.** These results indicate that βGBP induces apoptosis through modulation of tumor suppressor proteins. The apoptotic response observed in different cell lines following βGBP treatment is presented in Table 4a, while the effect of βGBP on p53 expression is summarized in Table 4b.

Antibacterial and Antifungal Activity of βGBP

The antibacterial activity of βGBP was evaluated against both Gram-positive and Gram-negative bacterial pathogens. βGBP exhibited inhibitory activity against *Escherichia coli, Staphylococcus aureus, Salmonella paratyphi, Pseudomonas aeruginosa, Bacillus subtilis*, and *Streptococcus pyogenes*. Among the tested organisms, maximum inhibition was observed against *Bacillus subtilis*. The zone of inhibition increased with increasing concentration of βGBP. Similarly, βGBP demonstrated significant antifungal activity against *Candida albicans, Aspergillus niger, Mucor* sp., *Rhizopus* sp., *Aspergillus flavus*, and *Aspergillus fumigatus*. The purified protein exhibited concentration-dependent inhibition against fungal pathogens, suggesting broad-spectrum antimicrobial potential. Representative antibacterial and antifungal inhibition zones produced by βGBP are shown in Figures 4a and 4b, respectively. Quantitative antibacterial and antifungal activities against the tested microorganisms are summarized in Tables 5a and 5b, respectively.

Amino Acid Composition of Purified βGBP

Amino acid composition analysis revealed the presence of both essential and non-essential amino acids in purified βGBP. Higher concentrations of valine, arginine, lysine, tryptophan, cysteine, and asparagine were observed when compared with standard values. HPLC purity analysis demonstrated that the

Table 3: Antiproliferative activity and IC_{50} values of βGBP against cancer cell lines.

Cell lines	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	IC_{50} µg/mL
HEPG2	72.507	77.134	92.549	80.057	88.837	40.84
EAC	54.327	57.428	50.692	40.895	34.775	44.75
MCF 7	46.646	50.693	61.213	56.884	64.345	80.46
A375	43.983	40.895	55.529	55.004	57.719	90.08
HELA	38.074	34.856	48.740	50.275	51.800	93.02
L929	78.112	73.276	71.347	67.824	64.022	97.19

Table 4a: Analysis of apoptotic cell death on cell line.

Cell lines	βGBP concentration (µg/mL)			
	Control	2	6	10
HEPG2	111	127	173	177
EAC	8	39	71	128
MCF 7	119	166	214	305
A375	98.88	89.86	82.67	66.70
HELA	1.12	10.13	17.32	33.29
L929	0.08	3.95	12.30	42.61

Table 4b: p53 expression of β-1,3 GBP.

Sample	Mean p53 expression
Untreated	9.81
Campothecin 15 µM standard	80.75
βGBP Treated	40.35

isolated βGBP possessed greater than 90% purity, indicating successful purification through affinity chromatography. The detailed composition of essential and non-essential amino acids in purified βGBP is presented in Supplementary Tables S1 and S2, respectively. A comparative graphical representation of the amino acid profile of βGBP is shown in Supplementary Figure S1.

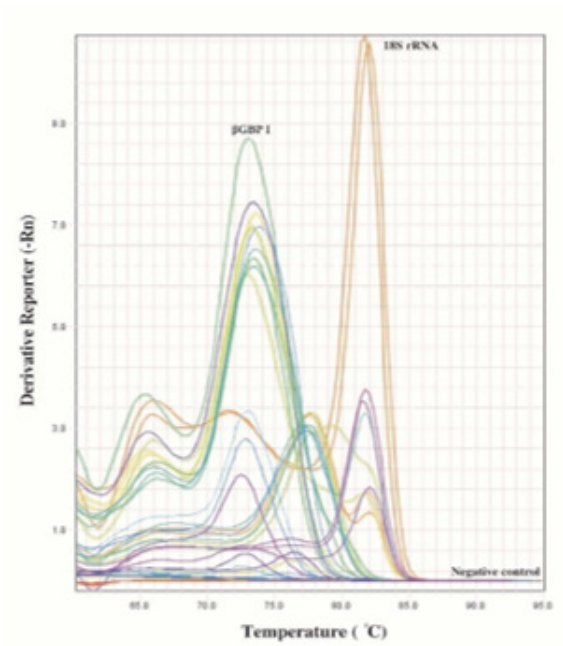
DISCUSSION

Marine-derived bioactive compounds have gained considerable attention in recent years due to their diverse pharmacological properties and potential therapeutic applications. Crustaceans possess highly efficient innate immune systems that rely on humoral and cellular defense mechanisms to combat invading pathogens. Among these immune-related biomolecules, β-1,3 glucan binding protein (βGBP) plays an important role in pathogen recognition, activation of immune responses, and antimicrobial defense. The present study investigated the relative gene expression, antiproliferative, apoptotic, antibacterial, and antifungal activities of βGBP isolated from the serum of *Emerita asiatica*.

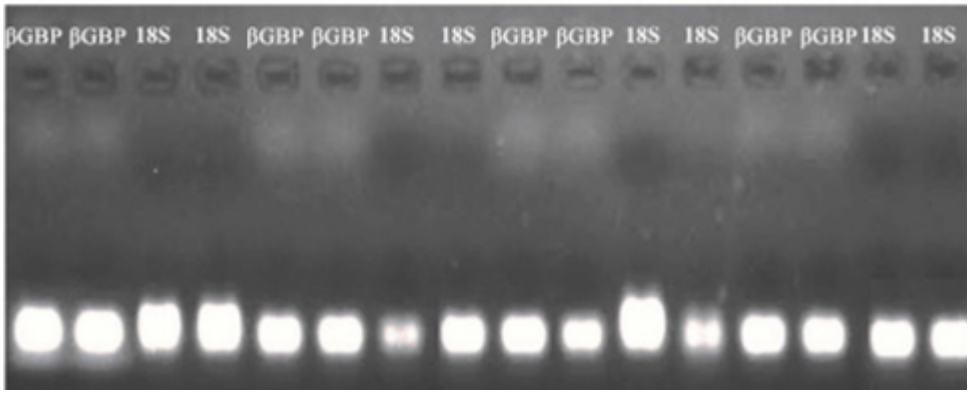
Nested PCR and qPCR analyses confirmed the presence and differential expression of βGBP genes in the serum of *E. asiatica*. Among the analyzed genes, βGBP I exhibited comparatively higher relative expression levels, suggesting its active involvement in crustacean immune defense. Previous investigations have reported that βGBP functions as a pattern recognition receptor capable of binding microbial polysaccharides and stimulating immune responses in marine arthropods (Mercurio and Shaw, 1991). The observed differential gene expression may indicate functional diversity among βGBP isoforms in response to environmental or pathogenic stress conditions. Similar findings were reported in earlier studies where crustacean βGBP genes were associated with antimicrobial and immunomodulatory activities.

The present investigation demonstrated significant antiproliferative activity of purified βGBP against multiple cancer cell lines. Among the tested cell lines, HepG2 cells exhibited the highest sensitivity with lower IC_{50} values compared to other cancer cells, while normal fibroblast cells showed comparatively higher resistance. These findings indicate selective cytotoxicity of βGBP towards cancer cells. Marine-derived proteins and polysaccharides have previously been reported to possess anticancer potential through inhibition of cell proliferation and induction of apoptosis. Priya and Ravichandran (2015) reported antiproliferative activity in marine crustaceans using MTT assay, supporting the present observations. Furthermore, studies by Shang *et al.* (2018) and Song *et al.* (2017) demonstrated that β-glucan-associated molecules could suppress carcinoma progression and enhance antitumor immune responses.

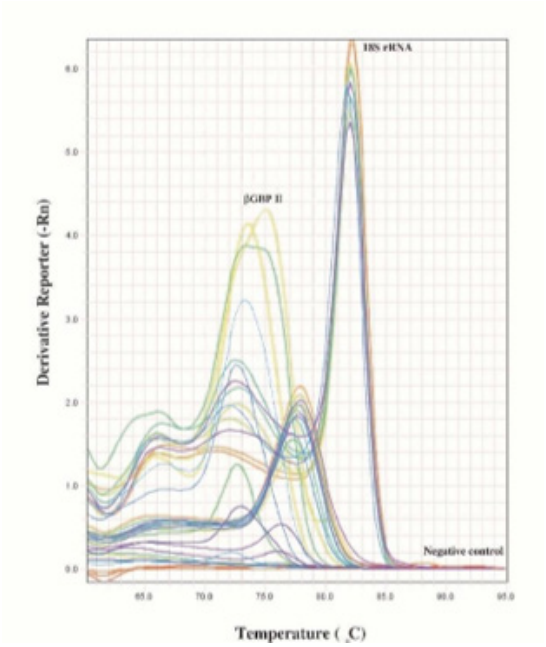
Morphological analysis and AO/EB dual staining confirmed apoptotic cell death in βGBP-treated HepG2 cells. Treated cells exhibited membrane shrinkage, chromatin condensation, and nuclear fragmentation, which are characteristic features of apoptosis. Flow cytometry analysis further demonstrated



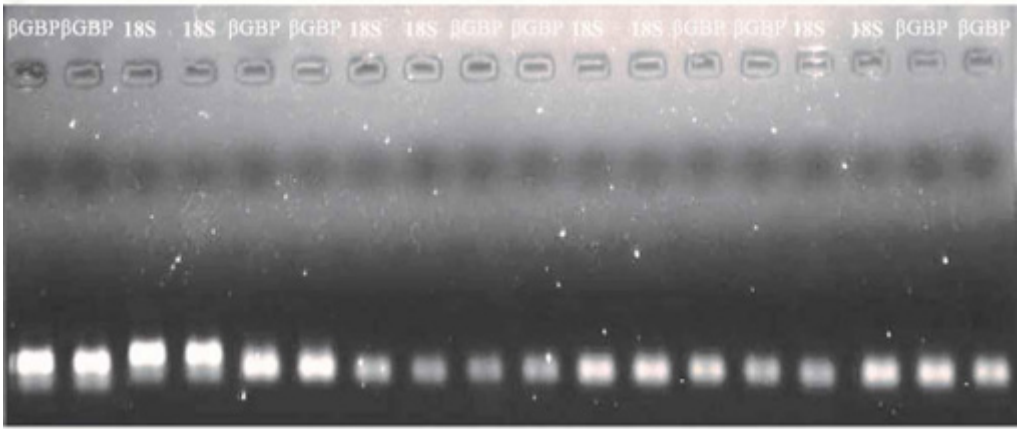
2a: βGBP I melt curve.



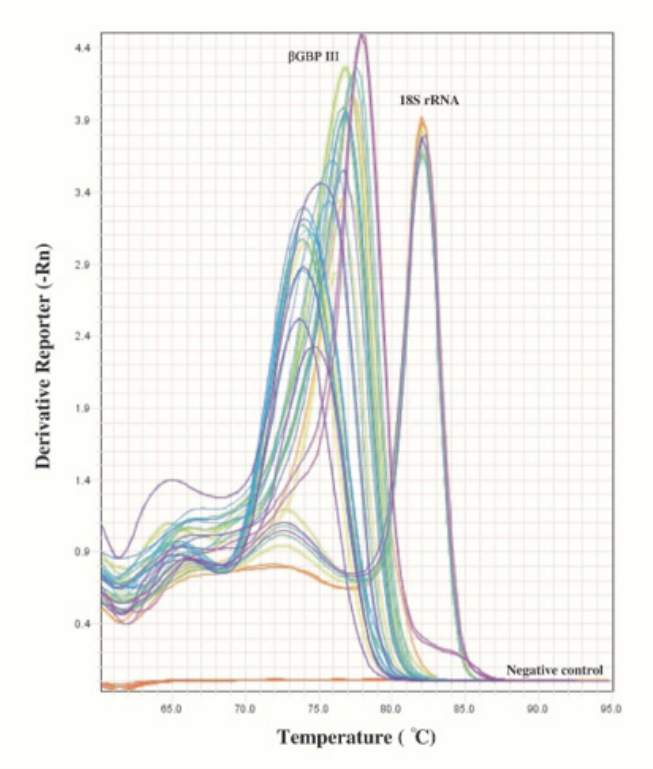
2b: βGBP I expression.



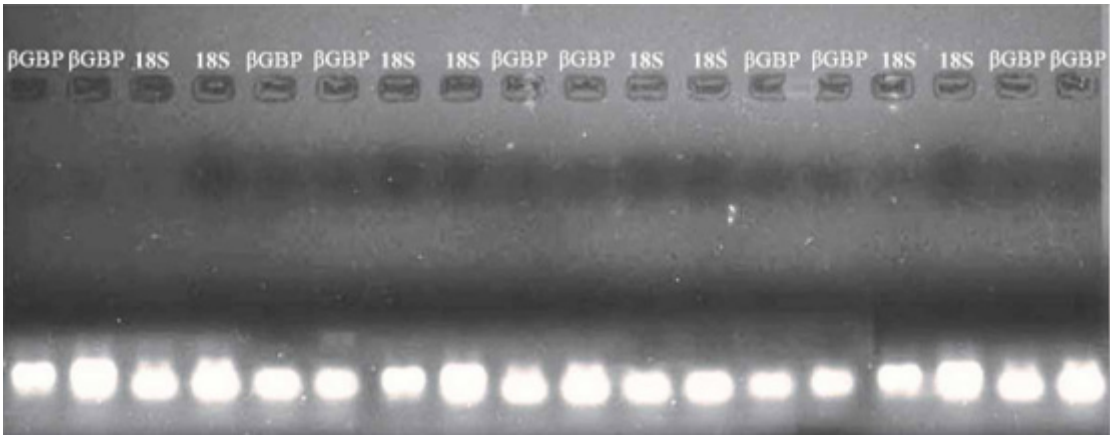
2c: βGBP II melt curve.



2d: βGBP II expression.



2e: βGBP III melt curve.



2f: βGBP III expression.

Figure 2a-2f: Relative gene expression and melt curve analysis of βGBP genes.

Table 5a: Bacterial Inhibition of βGBP.

Microorganisms/Sample	Zone of Inhibition in mm				
	20 µL	40 µL	60 µL	80 µL	Streptomycin (20 µg)
Serum					
E. coli	8	9	10	11	19
Staphylococcus aureus	8	9	10	11	17
Salmonella paratyphi		8	9	10	26
Pseudomonas aeruginosa		8	9	10	15
Bacillus subtilis			10	12	15
Streptococcus pyrogens			10	12	22

Table 5b: Antifungal activity of βGBP.

Microorganisms/Sample	Zone of Inhibition in mm				
	20 µL	40 µL	60 µL	80 µL	Ketocanzole (20 µL)
Serum					
Candida albicans	8	9	10	11	15
Aspergillus niger	8	9	10	11	17
Mucor sp.		9	10	11	26
Rhizopus sp.		9	10	11	17
Aspergillus flavus		9	10	12	19
Aspergillus fumigatus		9	10	12	23

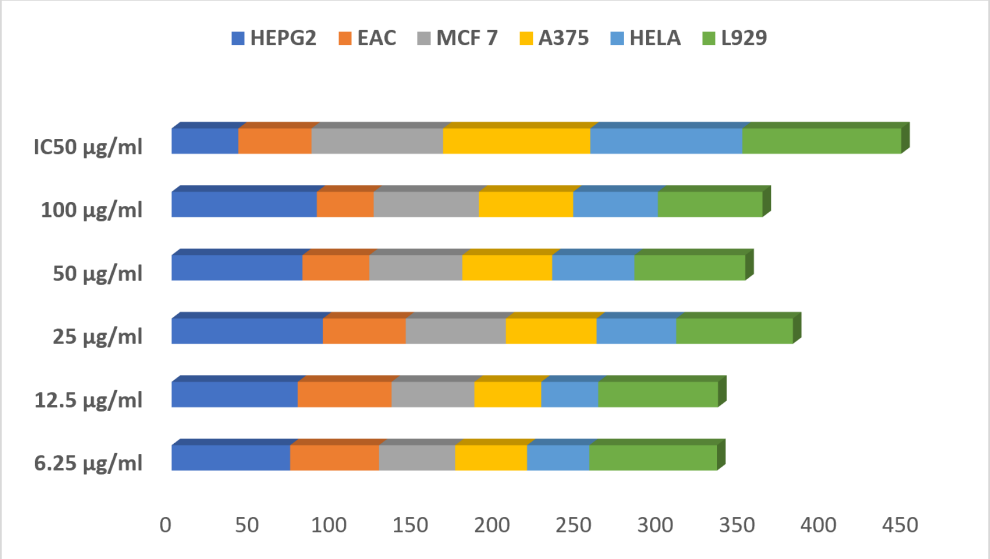


Figure 3: IC_{50} analysis of different cancer cell lines.

increased p53 expression in treated cells, indicating activation of p53-mediated apoptotic pathways. Tumor suppressor protein p53 plays a central role in regulating apoptosis, cell cycle arrest, and DNA repair mechanisms during cellular stress. Enhanced p53 expression observed in the present study suggests that βGBP induces apoptosis through modulation of intrinsic apoptotic pathways. Similar apoptotic induction by marine-derived proteins has been reported in HepG2 and other carcinoma cell lines.

In addition to anticancer activity, βGBP exhibited broad-spectrum antibacterial and antifungal properties against several pathogenic microorganisms. Significant inhibitory activity was observed against both Gram-positive and Gram-negative bacteria, as well as fungal pathogens including *Candida albicans* and *Aspergillus* species. Crustacean immune proteins are known to participate in microbial recognition and elimination through agglutination, opsonization, and activation of immune cascades. Previous studies have demonstrated that crustacean-derived antimicrobial peptides and lectin-like proteins contribute substantially

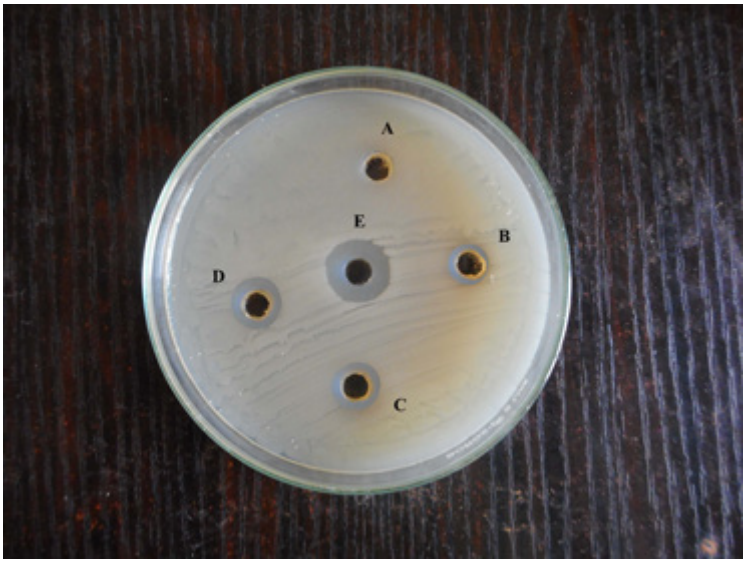


Figure 4a: Bacterial Inhibition of βGBP I- *Staphylococcus aureus*.

(ZONE A: 20 µL of sample, ZONE B: 40 µL of sample, ZONE C: 60 µL of sample, ZONE D: 80 µL of sample, ZONE E: 20 µg of Streptomycin).

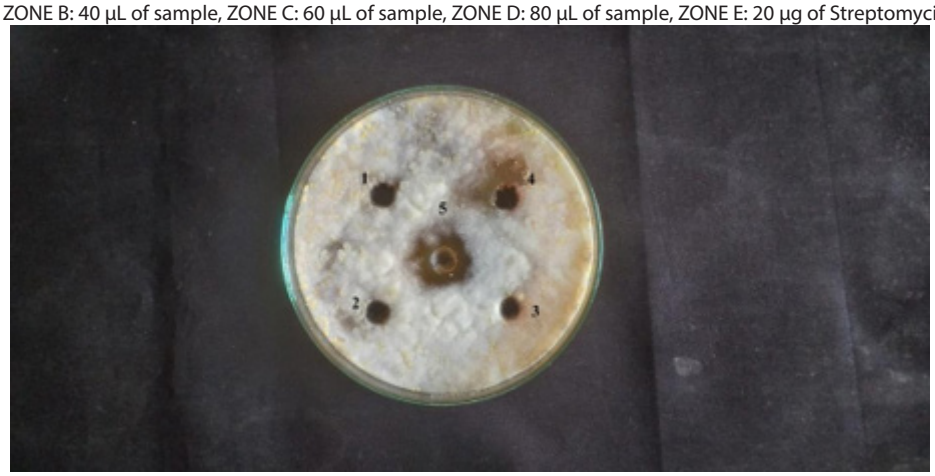


Figure 4b: Fungal Inhibition of βGBP I- *Aspergillus niger*.

(ZONE A:20 µL-sample, ZONE B: 40 µL-sample, ZONE C: 60 µL-sample, ZONE D: 80 µL-sample, ZONE 5: 20 µL of ketocanzole).

to nonspecific immune defense mechanisms. The observed antimicrobial activity in the present study further supports the role of βGBP as an important humoral immune factor in *E. asiatica*.

The amino acid composition analysis revealed the presence of essential and non-essential amino acids in purified βGBP, with relatively higher levels of valine, arginine, lysine, tryptophan, cysteine, and asparagine. The presence of these amino acids may contribute to the structural stability and biological activity of βGBP. HPLC analysis demonstrated high protein purity following affinity chromatography, confirming the successful isolation and purification of the protein from serum samples.

Overall, the present study demonstrates that βGBP isolated from *Emerita asiatica* possesses significant immunological, antimicrobial, and antiproliferative properties. The enhanced expression of p53 and induction of apoptosis in HepG2 cells highlight the therapeutic potential of βGBP as a marine-derived anticancer biomolecule. These findings provide scientific evidence supporting the biomedical importance of crustacean immune proteins and suggest that βGBP may serve as a promising candidate for future anticancer and antimicrobial drug development.

CONCLUSION

The present investigation demonstrated that β-1,3 glucan binding protein isolated from the serum of *Emerita asiatica* possesses significant antiproliferative, apoptotic, antibacterial, and antifungal activities. The enhanced p53 expression and apoptotic induction observed in HepG2 cells suggest that βGBP exerts its anticancer effect through activation of apoptosis-mediated pathways. Furthermore, the broad-spectrum antimicrobial activity indicates its important role in crustacean innate immunity.

The findings of this study emphasize the therapeutic potential of marine-derived βGBP as a promising bioactive molecule for

future anticancer and antimicrobial applications. Further studies involving purification, structural characterization, molecular pathway analysis, and *in vivo* evaluation may provide additional insights into its biomedical and pharmaceutical relevance.

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ABBREVIATIONS

βGBP: β-1,3 glucan binding protein; **ATC:** Anti-cancer activity; **proPO:** Prophenol oxidase; **ABT:** Antibacterial; **AGE:** Agarose gel electrophoresis; **cDNA:** Complementary DNA; **rRNA:** Ribosomal RNA; **qPCR:** Quantitative polymerase chain reaction; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **Ct:** Threshold cycle; **IC_{50}:** Fifty percentage of inhibitory concentration; **DMSO:** Dimethyl sulfoxide; **PBS:** Phosphate-buffered saline; **AA:** Amino acids; **HPLC:** High-performance liquid chromatography; **DEAE:** Diethylaminoethyl; **EAC:** Ehrlich Ascites Carcinoma; **EtBr:** Ethidium bromide; **NFW:** Nuclease-free water;

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

S. Eshwaran: Conceptualization, experimentation, manuscript preparation. S.S. Jayaraj: Investigation and data analysis. R. Thiagarajan: Technical support and validation. K.P. Dinakaran: Review and supervision.

SUMMARY

The present study investigated the relative gene expression, antiproliferative, apoptotic, antibacterial, and antifungal activities of β-1,3 Glucan Binding Protein (βGBP) isolated from the serum of *Emerita asiatica*. Molecular analysis using nested PCR and qPCR confirmed the presence and differential expression of βGBP genes I, II, and III, with βGBP I showing comparatively higher expression levels. The purified βGBP exhibited significant dose-dependent antiproliferative activity against various human cancer cell lines, particularly HepG2 and EAC cells, while demonstrating comparatively lower toxicity toward normal fibroblast cells.

Morphological observations, AO/EB dual staining, and flow cytometry analysis confirmed that βGBP induced apoptotic cell death through enhanced p53 expression in HepG2 cells. In addition, βGBP demonstrated broad-spectrum antibacterial and antifungal activity against several clinically important microbial pathogens. Amino acid composition analysis revealed the presence of both essential and non-essential amino acids, while HPLC analysis confirmed high purity of the isolated protein. Overall, the study highlights the biological and therapeutic significance of βGBP derived from *Emerita asiatica*.

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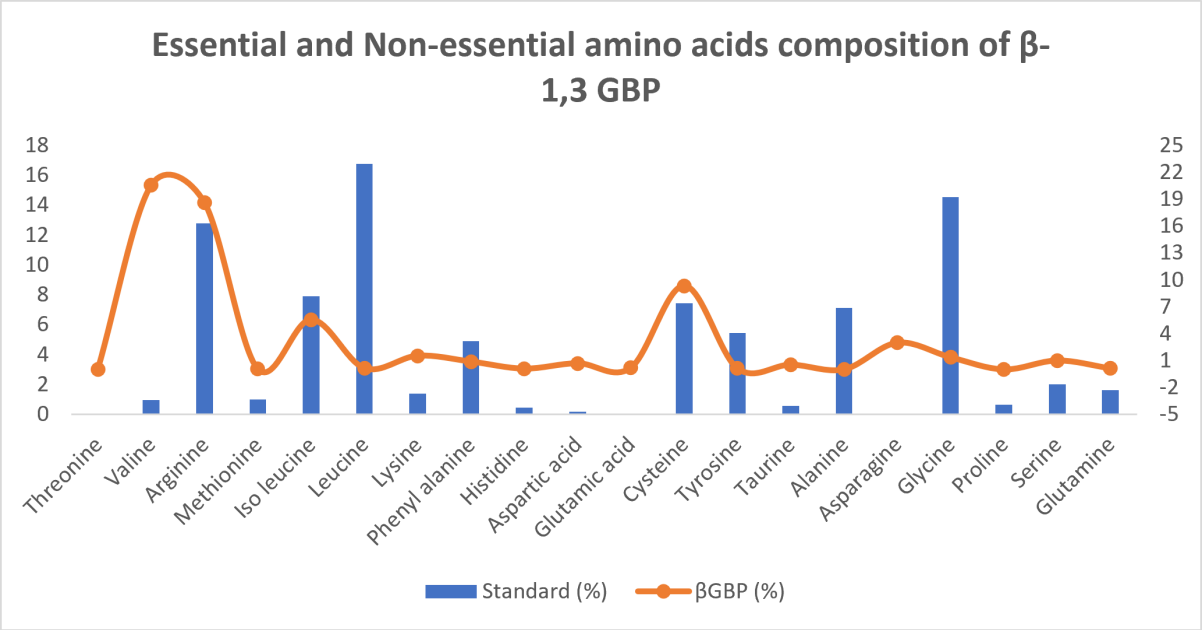

Supplementary Figure S1: Essential and Non-essential amino acids composition of β-1,3 GBP.

Supplementary Table S1: Essential amino acid composition of purified βGBP.

Essential Amino Acids	Standard (%)	βGBP (%)
Threonine	0.001	0.001
Valine	0.980	20.536
Arginine	12.780	18.624
Methionine	0.991	0.067
Iso leucine	7.890	5.577
Leucine	16.77	0.156
Lysine	1.378	1.540
Phenyl alanine	4.890	0.889
Histidine	0.445	0.101
Tryptophan	0.675	10.550

Supplementary Table S2: Non-essential amino acid composition of purified βGBP.

Non- Essential Amino acids	Standard (%)	βGBP (%)
Aspartic acid	0.191	0.701
Glutamic acid	0.0332	0.201
Cysteine	7.44	9.345
Tyrosine	5.44	0.122
Taurine	0.564	0.538
Alanine	7.110	0.011
Asparagine	0.023	2.989
Glycine	14.55	1.359
Proline	0.645	0.006
Serine	2.020	1.004
Glutamine	1.610	0.122