Journal of Health and Rehabilitation Research 2791-156X JHRR

For contributions to JHRR, contact at email: editor@jhrlmc.com

Original Article

In-Vitro Evaluation of Cytotoxicity and Antiinflammatory Potential of Hypnea Musciform (Rhodophyta) Collected from the Coastal Area of Karachi, Pakistan

Wasif Iqbal[1]*, Nadeem Hafeez Khokhar[2], Abdur Rehman[2], Shafaq Noori[1], Muhammad Imran[1], Abdul Samad Khan[3]

[1]Jinnah Sindh Medical University Karachi.
[2]Hamdard University Dental Hospital Karachi
[3]College of Dentistry
Corresponding Author: Wasif Iqbal; Email: wasif.vp@gmail.com

Conflict of Interest: None.

Iqbal W., et al. (2024). 4(1): DOI: https://doi.org/10.61919/jhrr.v4i1.399

ABSTRACT

Background: The extensive littoral zones of Karachi, Pakistan, are home to a diverse marine phytoplankton population, including the underutilized Hypnea musciformis. This red algae is an unexplored potential source of bioactive compounds for pharmacological use.

Objective: This study aimed to evaluate the cytotoxicity and anti-inflammatory potential of the aqueous extract of Hypnea musciformis collected from the coastal areas of Karachi.

Methods: Hypnea musciformis was collected from November 2019 to February 2021, with environmental parameters such as seawater temperature, pH, and salinity recorded. The collected samples were dried, powdered, and extracted in water. The cytotoxicity was assessed using a brine shrimp lethality test across a range of concentrations (1, 10, 100, and 1000 µg/ml), determining the LC50 value. Anti-inflammatory activity was evaluated via a membrane stabilization method on human red blood cells (HRBC), comparing the effects with the standard drug Diclofenac at concentrations ranging from 100 to 200 µg/ml.

Results: The brine shrimp lethality assay showed a dose-dependent increase in mortality with the highest concentration exhibiting 83% mortality, yielding an LC50 value of 550 µg/ml. The anti-inflammatory assay demonstrated that the aqueous extract of Hypnea musciformis provided a concentration-dependent protection with a maximum protection percentage of 78.64% at 200 µg/ml, while the control substance, Diclofenac, displayed higher efficacy.

Conclusion: The aqueous extract of Hypnea musciformis possesses significant cytotoxic and anti-inflammatory activities, suggesting its potential as a source of natural compounds for therapeutic purposes. However, further studies are required to elucidate the mechanisms of action and to confirm the safety and effectiveness of the extract in clinical applications.

Keywords: Hypnea musciformis, cytotoxicity, anti-inflammatory, brine shrimp lethality assay, membrane stabilization, bioactive compounds, marine phytoplankton.

INTRODUCTION

The increasing prevalence of heterogeneous tumor and cancer cell populations has intensified the demand for multi-targeted therapeutic strategies aimed at enhancing the pharmacological efficacy of treatments for cancer and inflammation (1). Despite the significant advancements in anti-cancer and anti-inflammatory medications, their use is often marred by substantial side effects (2), underscoring the necessity for safer, more effective alternatives. Historically, herbal plants have been harnessed as medicines, offering low-cost, lower-risk treatment options compared to their synthetic counterparts (3). However, in the past few decades, the role of herbal medicines in the research and development landscape of pharmacotherapy has been relatively secondary, despite their potential health benefits (4). This is particularly true in industrialized nations, where plants and natural compounds have been increasingly considered as supplementary health measures (5). Among the vast biodiversity of potential medicinal sources, marine species stand out for their rich phytochemical profiles, having been extensively utilized in traditional healing practices (6).

Journal of Health and Rehabilitation Research 2791-156X JHRR

The exploration of Pteridophytic plants for their medicinal properties is of significant interest, particularly in the assessment of cytotoxicity, which serves as a critical indicator of a plant's potential adverse effects on normal cells (7). Such evaluations are essential to ensure that traditional remedies can transition safely into clinical applications, necessitating rigorous preclinical and clinical testing (8). Indeed, traditional remedies exhibiting cytotoxic properties hold promise for development into novel anticancer therapies (9), highlighting the untapped potential of natural products in drug discovery and development—a field that has somewhat neglected these sources over the last several decades (10). The premise that natural compounds derived from plant extracts could offer potent anti-inflammatory benefits with minimal or no toxicological risks further supports the exploration of these natural resources (11).

Given Pakistan's rich algal flora, this investigation focuses on the aqueous extract of Hypnea musciformis collected from the coastal area of Karachi, aiming to assess its toxicity levels and in-vitro anti-inflammatory activity. This study is motivated by the need to explore safer, more effective therapeutic agents that can complement or even replace conventional treatments fraught with side effects. Through this research, we seek to contribute to the broader understanding of the pharmacological potential of marine-derived phytochemicals, positioning Hypnea musciformis as a candidate for further development in the fight against cancer and inflammation.

MATERIAL AND METHODS

The methodologies for the collection and evaluation of Hypnea musciformis, an intertidal red alga, were meticulously designed to ensure the validity and reliability of the results. Prior to the commencement of specimen collection, key environmental parameters such as sea water salinity, pH, and temperature were recorded using a refractometer, pH strips, and a thermometer, respectively, to ensure that the samples were collected under consistent environmental conditions. The collection phase was conducted over a span of sixteen months, from November 2019 to March 2021, along the coast of Buleji near Karachi, with particular attention paid to low tide periods to facilitate access to the intertidal algae.

Upon successful identification by the Department of Botany at the University of Karachi, the specimens of Hypnea musciformis, belonging to the Rhodophyceae family, were prepared for analysis. The collection process was performed with great care, where the algae were transported to the laboratory in clearly marked bags. The specimens were then subjected to thorough rinsing with fresh water, repeated three to five times, and subsequently laid out on nylon gauze to air dry in the shade, preventing any degradation of sensitive compounds.

For extraction, 10 grams of the dried algal powder were agitated in 100 milliliters of water using a mechanical shaker for a period of seven hours. The resultant mixture underwent centrifugation at 7000 revolutions per minute for fifteen minutes, after which the supernatant was filtered using a vacuum filtration system. The aqueous extracts thus obtained were then evaluated for toxicity using the standard brine shrimp lethality assay (12).

The assessment of the extract's toxicity involved a careful process of serial dilution, creating concentrations ranging from 1 mg/mL to 1 µg/mL. Post dilution, test containers were inoculated with ten nauplii and 1 mL of each concentration, and mortality rates were observed after a 24-hour period. The chronic LC50 value, which indicates the lethal concentration causing 50% mortality in the test organisms within 24 hours, was determined using the probit analysis method.

In addition to toxicity assays, the anti-inflammatory potential of the Hypnea musciformis extracts was investigated using the human red blood cell (HRBC) membrane stabilization method (13). Blood samples were collected and treated with Alsever's solution, and the packed cells obtained post-centrifugation were washed with isosaline to create a 10% v/v solution. The test mixture, consisting of HRBC solution, hyposaline, and phosphate buffer, was incubated with the extracts. Diclofenac served as the reference drug for comparison, and hemolysis was measured by the absorbance of the supernatant at 560 nm using a UV-Visible spectrophotometer.

All procedures involving the collection and handling of samples, as well as the experimental assays, were conducted with strict adherence to ethical guidelines. Data collected during the study was systematically recorded and analyzed using the Statistical Package for the Social Sciences (SPSS), version 25, ensuring that the analysis was performed with the most current methodologies available at the time of the study. The comprehensive approach taken in the methodological design of this study aimed to ensure that the findings on the cytotoxicity and anti-inflammatory potential of Hypnea musciformis would be both robust and reliable, contributing valuable insights into the pharmacological applications of this marine species.

RESULTS

The results from the brine shrimp toxicity assay reveal distinct mortality patterns between the control and standard groups. In the control group, where the pH was held constant at 7.1, brine shrimp mortality remained at a consistent 0% across all tested concentrations of the substance, as reflected by the minimal R-squared value of 0.0016, which implies virtually no correlation between substance concentration and mortality. In stark contrast, the standard group, at a lower pH of 6.5, showed a progressive

Journal of Health and Rehabilitation Research JHRR

increase in mortality with rising concentrations of the substance. This trend is quantitatively depicted by the R-squared value of 0.11, suggesting a modest linear relationship. Mortality in the standard group escalated with concentration, reaching a definitive 100% at the highest concentration. The temperature during the assay was controlled between 35 to 37 degrees Celsius, and salinity was measured between 80 to 140 ppm, which are essential parameters that may influence the outcome but are not directly correlated with mortality in the provided data.

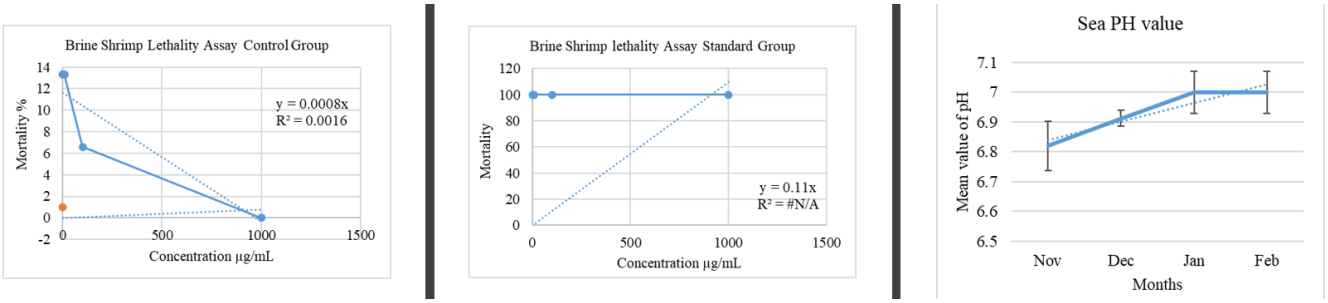

Figure 1 Brine Shrimp Lethality Assay

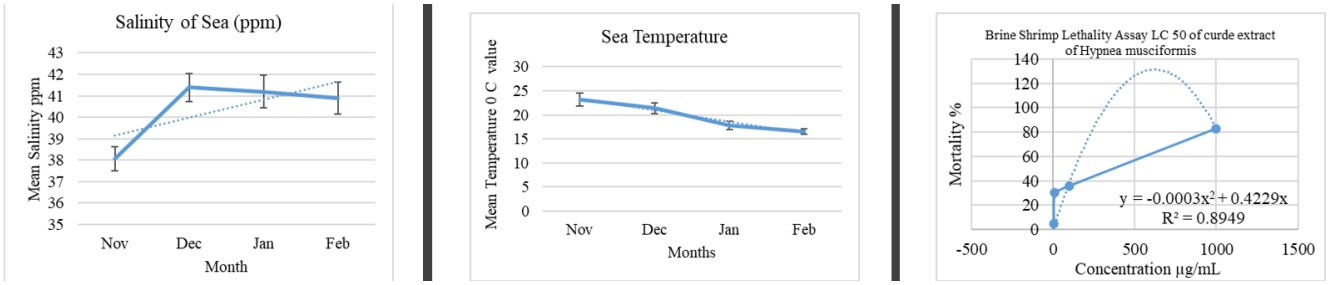

Figure 2 Variation of Salinity and Temperature Over Time

In the evaluation of Hypnea Musciformis extract's cytotoxicity through the Brine Shrimp Lethality Test, varied responses were observed at different concentrations (Table 1). The control group, which was treated with distilled water, exhibited a remarkably high survival rate of 96% at both 1 µg/mL and 10 µg/mL concentrations, with no mortality (LC50) value applicable as indicated by the dashes. At a concentration of 100 µg/mL, the survival rate slightly decreased to 93%, and a further increase in concentration to 1000 µg/mL resulted in a perfect survival rate of 100%. The mortality percentage in these groups ranged from 0% to 13.3%, reflecting the non-toxic nature of the control substance.

Contrastingly, the standard group exposed to Vincristine Sulphate demonstrated a starkly different outcome. There was a consistent 100% mortality rate across all concentrations from 1 µg/mL to 1000 µg/mL, indicating the high toxicity of the standard substance with an LC50 value firmly at 100 µg/mL.

Table 1: Brine Shrimp Lethality Test (LC50) of Hypnea Musciformis Extract

Plant Extract	Concentration (µg/mL)	Surviving Nauplii at 24 hrs	Total Survival Nauplii	Survival %	LC50 (µg/mL)	Mortality %
Control (Distilled H2O)	1	10	9	96	0	13.3
	10	10	9	96	-	13.3
	100	10	8	93	-	6.6
	1000	10	10	100	-	0
Standard (Vincristine Sulphate)	1	0	0	0	100	100
	10	0	0	0	-	100
	100	0	0	0	-	100
	1000	0	0	0	-	100
Hypnea Musciformis	1	10	8	93	550	5
	10	8	7	73	-	30
	100	2	3	63	-	36
	1000	0	0	16	-	83

The 'LC50 (µg/mL)' column represents the concentration of extract at which 50% mortality of nauplii is observed.
Dashes (-) indicate that the LC50 value is not applicable or not determined for those concentrations.
The 'Mortality %' column represents the percentage of nauplii that did not survive at each concentration.

Journal of Health and Rehabilitation Research JHRR

Table 2: Anti-Inflammatory Test for Hypnea Musciformis Extract

Concentration (µg/mL)	Standard (Diclofenac)	Hypnea Musciformis (Aqueous Extract)
	Protection %	Hemolysis %
100	62.99	37.01
120	75.54	24.46
160	81.81	18.19
200	91.11	8.89

For the Hypnea Musciformis extract, the survival rates at 24 hours post-exposure were 93%, 73%, 63%, and 16% for the respective concentrations of 1, 10, 100, and 1000 µg/mL. The LC50 value was determined to be 550 µg/mL, with mortality percentages increasing from 5% to 83% as the concentration rose, revealing a dose-dependent toxicity.

The anti-inflammatory potential of the extract was further assessed and compared with the standard anti-inflammatory agent, Diclofenac (Table 2). At 100 µg/mL, the standard showed a protection percentage of 62.99%, which increased in a dose-dependent manner, reaching 91.11% at 200 µg/mL. This correlated with a decrease in hemolysis percentage from 37.01% to 8.89%, respectively. The Hypnea Musciformis aqueous extract also displayed anti-inflammatory activity, albeit at a lower efficacy than Diclofenac. The protection percentages ranged from 44.69% at 100 µg/mL to 78.64% at 200 µg/mL, with corresponding hemolysis percentages decreasing from 55.31% to 21.36%, indicating a moderate anti-inflammatory effect of the algal extract.

These findings demonstrate the dual nature of Hypnea Musciformis extract as both a cytotoxic agent and an anti-inflammatory remedy, with its efficacy varying in relation to the concentration, thereby necessitating careful consideration in potential therapeutic applications.

DISCUSSION

The expansive Karachi coastline generates a significant biomass of marine phytoplankton, among which Hypnea musciformis, a species with considerable potential, remains largely underutilized (14). The survival and distribution of this species are intricately linked to their habitat, which is pivotal for the thriving of native Hypnea populations (15). During our study period from November 2019 to February 2021, a notable variation in environmental conditions was observed. Specifically, the mean sea temperature exhibited a decrease from 24°C to 16°C, which corresponded with the periods of peak algal growth noted in November and December (17). Concurrently, the pH of the sea water showed a gradual increase across the same months. It was evident that temperature is a critical factor influencing the production of Hypnea musciformis, with optimal growth rates occurring between 18-24°C, inversely related to biomass and solar irradiance (17). Salinity, another key determinant, showed a similar pattern of fluctuation, peaking between November and December, then tapering off through January and February. The optimal growth range of this algal species was identified in salinity levels of 25-40, with a marked decline beyond this range (18).

In assessing the cytotoxic potential of Hypnea musciformis, our findings revealed a direct correlation between extract concentration and lethality. The brine shrimp lethality assay, which employed varying concentrations of the aqueous extract, highlighted a lack of mortality in the control group and a stark contrast with a 100% mortality rate in the standard group treated with Vincristine sulphate. Interestingly, the survival rate was observed to be 5% at the lowest concentration of 1 µg/ml, escalating to an 83% mortality at the highest concentration of 1000 µg/ml, underscoring a dose-dependent lethality with an LC50 value at 550 µg/ml (19). This lethality could be attributed to the presence of bioactive phytochemicals such as alkaloids, flavonoids, and terpenoids, known to disrupt the biochemical and physiological functions of the nauplii by penetrating the plasma membrane (20, 21).

Comparatively, other studies have reported varying LC50 values for extracts from different plant species, demonstrating a range of cytotoxic effects against several cancer cell lines (19). For instance, Annona reticulata and Brassica oleraceae extracts exhibited LC50 values much higher than our findings, suggesting a lower cytotoxic effect (19). In contrast, extracts from Angiopteris evecta, Pyrrosia lanceolata, and Adiantum latifolium demonstrated LC50 values in a similar range to our study, indicative of their non-toxic nature (7).

Turning to the anti-inflammatory activity of Hypnea musciformis, our results indicated a concentration-dependent protective effect, aligning with a decrease in hemolysis as the extract concentration increased from 100 to 200 µg/ml. This suggests that the aqueous extract may stabilize the RBC membrane, thereby offering protection against hemolysis (22). A similar protective effect was reported by Silva M, et al., in 2022, wherein an ethanolic crude extract of P.chaba mirrored the efficacy of acetylsalicylic acid at 500 µg/ml (22).

Journal of Health and Rehabilitation Research JHRR 2791-156X

The study emphasized Hypnea musciformis as a species with potential therapeutic applications, given its cytotoxic and anti-inflammatory activities. While plant extracts with LC50 values greater than 1000 ppm are generally considered inactive, our extract's LC50 of 550 µg/ml positions it well within the range of biological significance. Furthermore, the membrane-mediated anti-inflammatory assay revealed a protective effect of up to 78.64%, reinforcing the extract's therapeutic viability. However, it is pertinent to acknowledge the limitations of this study, including the variability of environmental factors such as temperature and salinity, which may influence bioactive compound profiles. Future research should aim to standardize extraction procedures and investigate the specific bioactive components responsible for the observed biological activities. Additionally, in vivo studies and clinical trials will be critical in determining the safety and efficacy of Hypnea musciformis for therapeutic use.

CONCLUSION

The study concludes that Hypnea musciformis, sourced from Karachi's coast, exhibits promising cytotoxic and anti-inflammatory properties, with an LC50 of 550 µg/ml suggesting potential therapeutic value. The findings imply that this algal species could be a viable source of bioactive compounds for developing novel treatments. However, the implications of these results are tempered by the need for further research to validate the efficacy and safety of the extract in clinical settings, and to understand the influence of environmental variables on its bioactive constituents.

REFERENCES

1. Souza CÁ, de Oliveira BA, Santos SA, Batista FL, Andrade FR, Neto EJ, et al. Orofacial antinociceptive effect of sulphated polysaccharide from the marine algae Hypnea pseudomusciformis in rodents. Inflammopharmacology. 2019;27(3):261-9.
2. Sangeetha G, Vidhya R. In vitro anti-inflammatory activity of different parts of Pedalium murex (L.). Inflammation. 2016;4(3):31-6.
3. Subramaniyan V, Kayarohanam S, Kumar JA, Kumarasamy V. Impact of herbal drugs and its clinical application. Int J Res Pharm Sci. 2019;10(2):1340-5.
4. Khan T, Ali M, Khan A, Nisar P, Jan SA, Afridi S, et al. Anticancer plants: A review of the active phytochemicals, applications in animal models, and regulatory aspects. Biomolecules. 2019;10(1):47.
5. Akter M, Shohag S, Hossain MN. In-vivo pharmacological studies of hypnea musciformis found in the coast of Saint Martin Island of Bangladesh. Biores Commun-(BRC). 2023;9(01):1237-44.
6. Jegan SR, Manjusha WA. Screening of antioxidant, anticancer activity and GC-MS analysis of selected seaweeds from Kadiapattinam Coast, Kanyakumari. J Survey Fisher Sci. 2023;10 (1S):3749-56.
7. Ramya Roselin I, Catharin Sara S, Gayathiri M, Gnana Deepa Ruby R, Sujatha S. Evaluation of brine shrimp lethality of Adiantum latifolium lam. A medicinal fern. J Nat Remedies. 2020;20(1):176-9.
8. Sarah QS, Anny FC, Misbahuddin M. Brine shrimp lethality assay. Bangladesh J Pharmacol. 2017;12(2):186-9.
9. Suneka S, Manoranjan T. Brine shrimp lethality assay with selected medicinal plants extracts. Vingnanam J Sci. 2021;16(2).
10. Osman NI, Sidik NJ, Awal A, Adam NA, Rezali NI. In vitro xanthine oxidase and albumin denaturation inhibition assay of Barringtonia racemosa L. and total phenolic content analysis for potential anti-inflammatory use in gouty arthritis. J Intercult Ethnopharmacol. 2016;5(4):343.
11. Pangestuti R, Getachew AT, Siahaan EA, Chun BS. Characterization of functional materials derived from tropical red seaweed Hypnea musciformis produced by subcritical water extraction systems. J Appl Phycol. 2019;31:2517-28.
12. Gandhidasan R, Thamaraichelvan A, Baburaj S. Anti-inflammatory action of Lannea coromandelica by HRBC membrane stabilization. Fitoterapia. 1991;62(1):81-3.
13. Hussain SA, Saeed VA, Masood A. Economic seaweeds of Pakistan coast. Pak J Mar Biol. 2001;7(1).
14. Yong WT, Thien VY, Rupert R, Rodrigues KF. Seaweed: a potential climate change solution. Renew Sustain Energy Rev. 2022;159:112222.
15. Lian Y, Wang R, Zheng J, Chen W, Chang L, Li C, et al. Carbon sequestration assessment and analysis in the whole life cycle of seaweed. Environ Res Lett. 2023.
16. Ding L, Ma Y, Huang B, Chen S. Effects of seawater salinity and temperature on growth and pigment contents in Hypnea cervicornis J. Agardh (Gigartinales, Rhodophyta). BioMed Res Int. 2013;2013.
17. Karsten U. Seaweed acclimation to salinity and desiccation stress. In: Seaweed biology: Novel insights into ecophysiology, ecology and utilization. Berlin, Heidelberg: Springer Berlin Heidelberg; 2012. p. 87-107.
18. Waghulde S, Kale MK, Patil V. Brine shrimp lethality assay of the aqueous and ethanolic extracts of the selected species of medicinal plants. Multidiscip Digital Publish Instit Proceed. 2019;41(1):47.

Journal of Health and Rehabilitation Research 2791-156X JHRR

19. Verma S. Medicinal plants with anti-inflammatory activity. J Phytopharmacol. 2016;5(4):157-9.
20. Nerdy N, Lestari P, Sinaga JP, Ginting S, Zebua NF, Mierza V, et al. Brine shrimp (Artemia salina Leach.) lethality test of ethanolic extract from green betel (Piper betle Linn.) and red betel (Piper crocatum Ruiz and Pav.) through the soxhletation method for cytotoxicity test. Open Access Maced J Med Sci. 2021;9(A):407-12.
21. Yesmin S, Paul A, Naz T, Rahman AA, Akhter SF, Wahed MI, Emran TB, et al. Membrane stabilization as a mechanism of the anti-inflammatory activity of ethanolic root extract of Choi (Piper chaba). Clin Phytosci. 2020;6:1-10.
22. Silva MG, Hort MA, Hädrich G, Bosco LD, Vaz GR, Silva MM, et al. Anti-inflammatory and antioxidant effects of the microalga Pediastrum Boryanum in carrageenan-induced rat paw edema. Braz Arch Biol Technol. 2022;64.