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Full Length Article

Elemental Composition, Antioxidant, Anti-inflammatory and Anti-genotoxic Properties of *Nitophyllum punctatum*

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ABSTRACT

Objectives: Marine algae are important sources of nutritionally and pharmacologically important products. Here in the study, nutritional composition and biological activities of a marine red algae *Nitophyllum punctatum* were assessed. The present study aimed to analyze the nutritional composition and biological effects of the *N. punctatum.*

Methods: The marine algae *N. punctatum* was collected, shade dried and extracted using Soxhelet apparatus. The elemental composition was estimated using Atomic Absorption Spectroscopy; the anti-inflammatory effect was determined using Raw 264.7 macrophages. Genotoxicity was assessed by standard methods using *Allium cepa* root model.

Results: The elemental composition analysis revealed the presence of essential minerals such as calcium, magnesium, potassium, and iron, indicating the potential nutritional value of the algae. The extract demonstrated significant inhibition of Nitric oxide (NO) production and downregulated the expression of pro-inflammatory cytokines such as TNF-α and IL-6, suggesting its potential as an anti-inflammatory agent. Furthermore, the anti-genotoxic potential of the methanol extract was evaluated using the *Allium cepa* model, a well-established system for assessing genotoxicity. Onion root tips were treated with various concentrations of the extract, and the chromosomal changes were performed as indicators of genotoxicity. Results indicated a protective effect against Ethyl methyl sulfonate (EMS) induced genotoxicity in onion roots.

Conclusions: The findings suggest that the methanol extract of *N. punctatum* possesses potent anti-inflammatory and anti-genotoxic properties, which may be attributed to the presence of bioactive compounds. The study highlights the potential pharmacological importance of *N. punctatum* as a source of natural compounds with anti-inflammatory and anti-genotoxic activities.

1. Introduction

Marine algae, encompassing a diverse range of photosynthetic organisms, are pivotal to oceanic ecosystems. These include microscopic phytoplankton and larger macroalgae like seaweeds (El Gamal, 2010). Macroalgae, such as kelp and red, green, and brown algae, provide habitats and nourishment for marine life. They are vital in coastal ecosystems, contributing to biodiversity and shoreline protection (Roy et al., 2022). Additionally, marine algae are economically significant and used in food, pharmaceuticals, and biofuels (Mandalka et al., 2022). Their role in biogeochemical cycles, nutrient cycling, and as bioindicators of environmental changes underscore their ecological importance (Dini, 2023). As climate change impacts marine environments, understanding and conserving marine algae is crucial for maintaining ocean health and mitigating global climate effects.

In coastal areas marine algae are found attached to rocks and other

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hard substrata. They are primary producers and contain chlorophyll which is a source of many nutrients, high protein content, and a rich source of dietary fibers. Some algae are used as food sources and for the preparation of beverages but the higher content of toxic elements in algal food products like cadmium or mycotoxins, limit their use as direct dietary food sources. Algae also synthesize various bioactive compounds including pigments (Wynne and Bold, 1985); by virtue of these molecules, the marine algae also possess many pharmacological properties (Neushul, 1990). The isolation of these active components has paved the way for the discovery of new anticancer drug formulations. They can also be used for the preparation of biodiesel, bioethanol, biobutanol, and some hydrogen gases (Bulgariu and Bulgariu, 2012). The algae are classified into three different groups based on color and storage of food (Garson, 1989), Brown, Green, and Red algae (Rhodophyta).

Around ten thousand species are described as red algae, belonging to the class Rhodophyceae (Wehr et al., 2015). Gelatinous filaments, free filaments, and pseudo-parenchymatous are the most common forms. The most common red algae include Irish moss, Laver, Dulse, etc. Red Algae have great ecological importance as they meet 40–60 % of total global oxygen demand (KRAFT, 1981). They also constitute the main food source in Japan and in the North Atlantic regions (El Gamal, 2010). They are a source of dietary fibers and help to promote body metabolism. One of the important groups of red algae is the Corallines algae, and coralline from it has been used in bone replacement therapies and used as vermifuges in ancient times (Naveed, 2014).

Red algae are abundant reservoirs of phenolic bioactive secondary metabolites. The spectrum of these phenolic derivatives encompasses flavonoids, simple phenolic acids, and halogenated derivatives. These bioactive elements hold significant promise in various medical applications. Flavonoids exhibit potent antioxidant properties, phenolic acids demonstrate anti-inflammatory effects, while bromophenols present diverse applications, including antimicrobial, anti-tumor, and potential antiviral activities, offering avenues for innovative therapeutic developments (Naveed, 2014). Algal polysaccharides have the potential to be exploited as an effective, non-toxic free radical scavenger that can be used to prevent oxidative damage in tissues (Kwon and Nam, 2007).

Lakshadweep has a marine ecosystem with many important marine plants and animals (Khan, 2017). Among the marine algae found, those belonging family Rhodophyceae is found in abundance. Usually, a lot of seaweeds are present in Lagoon Island like Agatti, Kavaratti, Bangaram, and Minicoy (Kaliaperumal and James, 1993). In Lakshadweep, seaweeds are special and valuable and are used as food items traditionally and also for medicines for allergy in Lakshadweep (Kaliaperumal and James, 1993). However, a very limited number of scientific studies are available on the pharmacological studies of marine algae from Lakshadweep.

In the present study, we went on to analyze the nutritional composition and biological effects of an under-explored red algae *Nitophyllum punctatum*. A study by Malhotra and Cojandaraj (2024) indicated the in vitro antioxidant and anti-diabetic effects of *N. marginale*. Similarly, the initial studies by Sridevi et al. (2003) indicated that the *N. marginata* contain important nutrients and therefore important source of dietary elements. There were no further studies on the pharmacological effects of the genus. Hence, we selected the plant *N. punctatum* for further analysis of the nutritional content and biological activities.

2. Materials and methods

2.1. Collection

The red algae of *Nitophyllum punctatum* (Fig. 1) belonging to the Delesseriaceae family were collected from Kavaratti island of Lakshadweep (10.5593 N, 72.6358 E). The alga was identified by Dr. Vinod K., Central Marine Fisheries Research Institute, Calicut, Kerala.

Fig. 1. *N. punctatum* plant.

2.2. Extraction of red algae

The methanol extract was obtained from powdered *N. punctatum* using soxhelet extraction. The resulting extract was then filtered to remove solid residues, and the methanol extract was concentrated using a rotary evaporator or freeze-drying technique. The concentrated extract was weighed, and appropriate dilutions were prepared using dimethyl sulfoxide (100 mg/mL) as the solvent for subsequent experiments.

2.3. Phytochemical and trace element analysis

Total phenol and flavonoid contents were estimated using the methods reported by Sidhic et al. (2023). Total polyphenol content was estimated using Folin-Ciocalteau reagent. The total flavonoid content in the extract was quantified by aluminum chloride ($AlCl_3$) method. The atomic absorption spectroscopy (Analyst 200, Perkin Elmer) and ICP-OES (Aveo 200, Perkin Elmer) was used to quantify the trace elements in the sample (El Hosry et al., 2023).

2.4. In vitro antioxidant activities

The in vitro antioxidant activities of the *N. punctatum* were evaluated using the hydrogen peroxide (H_2O_2) scavenging assay and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays (Meryem et al., 2023). The nitric oxide scavenging assay was used to elucidate the preliminary anti-inflammatory effects (Narayanankutty et al., 2023). The optical density of these treatments was estimated and subsequently percentage of inhibition was calculated and IC_{50} value was determined.

2.5. Inhibition of LPS mediated Raw 264.7 macrophage activation

To evaluate the anti-inflammatory potential, RAW 264.7 cells, a murine macrophage cell line, were cultured in DMEM media containing 10 % fetal bovine serum (FBS) and Pen-strep combination of antibiotics. The methanol extract of *N. punctatum* was treated prior to the LPS-stimulation in Raw 264.7 cells at varying concentrations, with appropriate controls. Subsequently, inducing an inflammatory response was achieved by stimulating the cells with lipopolysaccharide (LPS). After 24 h of incubation, the media and cells were collected to assess the efficacy of *N. punctatum*'s anti-inflammatory properties, encompassing the measurement of nitric oxide (NO) production inhibition and the evaluation of pro-inflammatory cytokine levels, such as TNF-α and IL-6.

2.6. Genotoxic and anti-genotoxic activities

The genotoxicity analysis of *N. punctatum* extracts utilizing the *Allium cepa* model was carried out through a meticulously designed

methodology (Dutta et al., 2018). Plant growing plates were set up, housing cotton soaked in distilled water and laden with various concentrations of the algal extract. Onion bulbs were strategically placed on the cotton, and the plates were incubated in darkness for a 72-hour period, facilitating the necessary exposure and interaction. Subsequent to this incubation, each onion root was carefully excised for thorough genotoxicity assessment.

Concurrently, an anti-genotoxicity assessment was conducted by subjecting different concentrations of the algal extract to treatment with ethyl methyl sulfonate (EMS). Following a similar incubation and excision procedure after 72 h of treatment, the excised root tips underwent a series of steps, including fixation in Carnoy's fixative, hydrolysis using 1 N hydrochloric acid, and staining with toluidine blue. These treated root tips were then gently squashed to create thin preparations, allowing for detailed examination under a microscope equipped with a 40x objective lens, providing a magnification of 400x. This microscopic analysis was crucial for scrutinizing chromosomal characteristics and discerning any aberrations or irregularities.

2.7. Statistical analysis

The results obtained from the in vitro studies were expressed as the mean ± standard deviation based on three independent sets of experiments, each performed in triplicate. For the genotoxicity and anti-genotoxicity assays, a total of 20 bulbs per treatment were used, and a minimum of 1000 cells were counted for analysis. Statistical analysis was conducted using one-way ANOVA, followed by Tukey-Kramer multiple comparison tests, using GraphPad Prism software version 7.0 (Windows, GraphPad Software, Boston, Massachusetts USA).

3. Results

3.1. Mineral and heavy metal composition using atomic absorption spectroscopy

The mineral composition of the *N. punctatum* was evaluated by atomic absorption spectrometry (AAS) technique. The result from quantitative analysis of the algal extract constituents are presented in Table 1. The concentrations of various metals and elements were measured; Copper was found at 1.70 ± 0.10 ppm, Calcium at 2241.5 ± 5.81 ppm, Zinc at 21.1 ± 1.6 ppm, and Manganese at 19.1 ± 1.6 ppm. Iron had a concentration of 19.1 ± 1.6 ppm, Phosphorus was detected at 2067.5 ± 9.1 ppm, Manganese at 1266.7 ± 8.4 ppm, and Zinc at 140.0 ± 2.0 ppm. Selenium was found at 1.54 ± 0.10 ppm, and Cobalt at 27.6 ± 2.2 ppm.

3.2. Phenol and flavanoid analysis

The results indicated that moderate amounts of phenolic compounds (47.5 ± mg GAE/g) are present in the tested red algal species *N. punctatum*. The flavonoid content of the extract was found to be lower than the phenolic content with an estimate of 20.4 ± mg GAE/g.

Table 1
The trace element concentrations of *N. punctatum* extract analyzed by AAS technique.

Mineral	Quantity (ppm)
Copper	1.70 ± 0.10
Calcium	2241.5 ± 5.81
Zinc	21.1 ± 1.6
Manganese	19.1 ± 1.6
Iron	19.1 ± 1.6
Phosphorus	2067.5 ± 9.1
Manganese	1266.7 ± 8.4
Zinc	140.0 ± 2.0
Selenium	1.54 ± 0.10
Cobalt	27.6 ± 2.2

3.3. In vitro antioxidant activity

The antioxidant effect of the extract was assessed in terms of the different radical scavenging activities. The results indicated that the extract dose-dependently scavenge the DPPH radicals and extract showed an IC_{50} value of 57.5 ± 3.11 mg/mL. Likewise, the scavenging of hydrogen peroxide scavenging assay showed significant radical scavenging and subsequently demonstrated to have an IC_{50} value of 74.7 ± 2.44 μg/mL. The anti-inflammatory efficacy was reported in terms of the nitric oxide scavenging potentials. The extract had strong antiinflammatory efficacy with an IC_{50} value of 18.3 ± 1.08 μg/mL.

3.4. In vitro anti-inflammatory activity

The antiiflammatory efficacy was further confirmed using cell culture models. The effects of *N. punctatum* (NP) on lipopolysaccharide (LPS)-induced macrophage (Raw 264.7) activation and cytokine release were assessed by measuring levels of IL-1β, IL-6, TNF-α, and NO. The untreated control group showed basal levels of these markers with IL-1β at 52.4 ± 1.4 pg/mg protein, IL-6 at 66.7 ± 4.2 pg/mg protein, TNF-α at 92.4 ± 4.7 pg/mg protein, and NO at 4.8 ± 0.30 μM/mg protein. Upon LPS treatment, there was a significant increase in the levels of all markers, indicating macrophage activation. IL-1β rose to 785.1 ± 19.7 pg/mg protein, IL-6 to 991.3 ± 15.0 pg/mg protein, TNF-α to 558.3 ± 17.2 pg/mg protein, and NO to 48.5 ± 1.3 μM/mg protein. Treatment with NP at 1.0 μg/mL resulted in a substantial reduction in the levels of these markers compared to the LPS alone group. IL-1β decreased to 511.3 ± 18.4 pg/mg protein ($P < 0.01$), IL-6 to 655.1 ± 10.8 pg/mg protein ($P < 0.01$), TNF-α to 404.4 ± 18.1 pg/mg protein ($P < 0.05$), and NO to 39.8 ± 0.8 μM/mg protein ($P < 0.05$). At a higher concentration of NP (2.5 μg/mL), the reductions were more pronounced: IL-1β levels dropped to 375.6 ± 15.1 pg/mg protein ($P < 0.01$), IL-6 to 501.7 ± 14.3 pg/mg protein ($P < 0.01$), TNF-α to 282.3 ± 14.5 pg/mg protein ($P < 0.01$), and NO to 30.7 ± 1.2 μM/mg protein ($P < 0.05$). The highest concentration of NP (5.0 μg/mL) produced the most significant decreases across all markers. IL-1β levels were reduced to 259.7 ± 10.9 pg/mg protein ($P < 0.001$), IL-6 to 327.4 ± 17.1 pg/mg protein ($P < 0.001$), TNF-α to 177.2 ± 17.0 pg/mg protein ($P < 0.001$), and NO to 24.4 ± 1.2 μM/mg protein ($P < 0.01$). These results demonstrate that *N. punctatum* effectively inhibits LPS-induced activation and cytokine release in macrophages in a dose-dependent manner (Table 2).

3.5. Genotoxicity analysis in Allium cepa model

The extract was found to be safer and non-genotoxic at concentrations up to 5 mg/mL and 10 mg/mL (Table 3). In contrast, treating *A. cepa* cells with EMS, which is a known genotoxicity inducer in cells, resulted in mutagenic responses over a 72-hour period. The frequency of cells displaying aberrations was quantified at 0.93 %. Moreover, the mitotic index significantly decreased in the EMS-treatment group.

Table 2
Effect of *N. punctatum* against LPS-mediated cytokine release (pg/mg protein) NO production (μM/mg protein).

	IL-1β	IL-6	TNF-α	NO
Untreated	52.4 ± 1.4	66.7 ± 4.2	92.4 ± 4.7	4.8 ± 0.30
LPS alone	785.1 ± 19.7	991.3 ± 15.0	558.3 ± 17.2	48.5 ± 1.3
NP 1.0 μg/mL	511.3 ± 18.4**	655.1 ± 10.8**	404.4 ± 18.1*	39.8 ± 0.8*
N 2.5 μg/mL	375.6 ± 15.1**	501.7 ± 14.3**	282.3 ± 14.5**	30.7 ± 1.2*
NP 5.0 μg/mL	259.7 ± 10.9***	327.4 ± 17.1***	177.2 ± 17.0***	24.4 ± 1.2**

(The significance is indicated as * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$).

Table 3
The in vitro genotoxic impact of the methanolic extract of *N. punctatum* was assessed and quantified as the percentage incidence in the *Allium cepa* root tips.

Treatment group	Total dividing cells	Mitotic index (%)	Sticky chromosomes	Distributed spindles & Disoriented chromosomes	Fragments	Micronuclei	Frequency Of aberrant cells (%)
Untreated	545	10.89	2	1	0	0	0.55
NP (5 mg/mL)	537	10.74	2	1	2	0	0.93
NP (10 mg/mL)	529	10.57	1	1	3	0	0.95

Interestingly, co-administration of methanolic extracts provided protection against EMS-induced microscopic changes in mitotic machinary of *A. cepa* (Table 4).

4. Discussion

Plants are important sources for bioactive secondary metabolites that are important pharmacological and nutritional agents (Uddin et al., 2019). Many of such plants are present in the diverse ecosystems of the land and known for their values in traditional and folk medicines (Mahmud et al., 2017; Muzammil et al., 2022). Several marine plants and marine sea weeds are also reported to have strong biopharmaceutical potentials. The algal extract widely used in Chinese medicine for treatment of various human diseases. Seaweeds are considered to be rich source of antioxidants. The anti-oxigenic activity of red algae are lower in comparison to brown algal species (Fujimoto and Kaneda, 1984). Previous studies reported that bioactive properties of read algae extract include antiviral, antifungal, anti-microbial, anti-diabetic and anti-tumour properties. Red alga *Plocamium* and *Chondrococcus* secretes polyhalogenated monoterpenes which are attributed to the antimicrobial and antitumor activity exhibited (Blunt et al., 2004). The industrial uses of the algal extract are predominantly confined on its insecticidal potentials. Dried extract of *N. punctatum* is used as a high potential larvicidal agent against the mosquito larvae (Elbanna and Hegazi, 2011).

The present study identified the presence of useful trace elements in *N. punctatum*. The trace elements, such as zinc, selenium, and copper, are crucial for maintaining health (Mehri, 2020). They function as antioxidants, protecting cells from damage, as well as have metabolic and pharmacological roles in various cellular processes (Zheng, 2020). Apart from these trace elements, the *N. punctatum* had higher phenolic and flavonoid contents. Phenolic compounds are importance agents that act as chain breaking antioxidants (Mittal and Raghavarao, 2018). The present study revealed that the phenolic (47.5 ± 1.55 mg GAE/ g) content was moderately low concentration. Previously, it has been reported that low concentration of phenolic compounds have been detected from the genus Gracilaria (Chen et al., 2013). Major phenolic compounds are expected to be bromo-chloro derivative and subsequently have stronger effects in the *Gracillaria* species (Huang and Wang, 2004). In the present study we expect the presence of phenolic compounds which are the derivatives of bromo-chloro halides, whose exact chemical nature needs to be ascertained in future studies.

In the present study the result of antioxidant assay revealed significant radical scavenging abilities against DPPH and hydrogen peroxide radicals. In the previous studies, *Gracilaria* showed of moderate to weak antioxidant potential (Chen et al., 2013). Similar studies on Chinese algae (Zhang et al., 2017), and Mexican algae (Zubia et al., 2009) reported antioxidant property under in vitro studies. In vivo antioxidant studies highlighted thhat chloroform extract derived from *Gracilaria blodgettii* Harvey has significant antioxidant potentials (Piao et al., 2014). This extract emerged as one of the most effective in mitigating oxidative and nitrosative stress induced in leucocytes. In other reports, the DPPH assay was used to test the inhibition of radical scavenging activity by the ethanolic and methanolic extract *G. birdiae* and *G. cornea* at different concentration, the result indicate that the high scavenging activity of ethanolic extract of *G. birdiae* (Huang and Wang, 2004).

Further, the anti-inflammatory activities of the algal extract were evident from nitric oxide radical scavenging and Raw 264.7 cells. Previous studies by Coura et al. (2015)) indicated a sulfated polysaccharide fraction extracted from Gracilaria cornea exhibits anti-inflammatory activity by inhibiting the release of histamine, blocking migration of neutrophils, and reducing vascular permeability. The compound R-phycoerythrin derived from various red seaweeds demonstrated substantial anti-inflammatory properties (Lee et al., 2017). Hence, the anti-inflammatory properties of the plant may be helpful to yield novel antioxidant and anti-inflammatory compounds in future.

Further, our study found no notable genotoxicity at various concentrations of the algal extracts. Conversely, exposure to EMS, a genotoxic agent, triggered significant genotoxic effects in allium cells, including sticky chromosomes, mitotic spindle irregularities, and breakage in *Allium cepa*. On contrary, the pre-treatment with the algal extracts protected against the EMS-induced genotoxicity in *A. cepa* root cells. Furthermore, our investigation revealed elevated levels of polyphenols and flavonoids in the algal extract, suggesting their potential role in conferring protection against free radicals and showcasing anti-inflammatory properties. This not only adds to our understanding of novel bioactive compounds but also hints at their potential for antioxidant, genotoxicity, and anti-inflammatory effects.

5. Conclusions

In conclusion, the methanol extract obtained from the red algae *N. punctatum* exhibits promising antioxidant, anti-inflammatory and anti-genotoxic effects. The elemental composition analysis reveals the presence of essential minerals, highlighting its potential nutritional value. The extract demonstrates significant inhibition of nitric oxide production and downregulation of pro-inflammatory cytokines, indicating its anti-inflammatory potential. Moreover, using the Allium cepa model, the extract shows a dose-dependent reduction in DNA damage, indicating its anti-genotoxic activity. Overall, the research on the elemental composition, anti-inflammatory, and anti-genotoxic properties of the methanol extract from *N. punctatum* sheds light on its pharmacological importance and paves the way for further investigations and applications in the fields of pharmaceuticals, nutraceuticals, and

Table 4
The safeguarding influence of the methanolic extract of *N. punctatum* was investigated against EMS-induced chromosomal aberrations in the *Allium cepa*.

Treatment group	Total dividing cells	Mitotic index (%)	Sticky chromosomes	Distributed spindles & disoriented chromosomes	Fragments	Micronuclei	Frequency Of aberrant cells (%)
Normal	510	10.19	2	1	1	0	0.79
EMS	383	7.65	37	18	21	18	24.58
NP (5 mg/mL)	552	11.04	20	21	12	10	11.41
NP (10 mg/mL)	522	10.44	16	8	11	7	8.05

functional foods.

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CRediT authorship contribution statement

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Declaration of competing 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.

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