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Original Article

Evaluation of Antioxidant Potential and Cytotoxic Effect of Iron Oxide Nanoparticles Synthesized from Red Seaweed *Gracilaria corticata*

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ABSTRACT

Background: Marine algae have received increasing attention over the past two decades because of their rich nutritional composition and diverse medicinal properties. At the same time, nanoparticles have emerged as promising tools for various therapeutic applications. However, the use of *Gracilaria corticata* (*G. corticata*), a red seaweed, for the synthesis of iron oxide nanoparticles (IONPs) remains largely unexplored. Therefore, the present study aimed to synthesize IONPs using the aqueous extract of G. corticata and evaluate their antioxidant and anticancer potential. **Materials and Methods:** Nutritional profiling of *G. corticata* by standard proximate analysis. Synthesis of IONPs and characterization by UV-visible Spectroscopy, FTIR and XRD analysis. Assessing antioxidant potential against DPPH, ABTS, FRAP, H_2O_2, and, Nitric Oxide radical inhibition assays and cytotoxic effect on A549 lung cancer cells. **Results:** Proximate analysis of the aqueous extract of *G. corticata* showed the presence of moisture (9.52%), ash (13.76%), carbohydrates (57.40%), protein (13.39%), fiber (3.18%), and lipids (2.75%). The synthesized IONPs exhibited strong dose-dependent antioxidant activity, with more than 85% radical scavenging observed at 50 µg/mL across all assays. In addition, the nanoparticles demonstrated dose-dependent cytotoxicity against A549 lung cancer cells, with an IC_{50} value of 71.3 µg/mL. **Conclusion:** This study highlights that *G. corticata*-mediated IONPs may serve as promising candidates for treatment of lung cancer.

Keywords: Antioxidant activity, Cytotoxicity, *Gracilaria corticata*, Iron oxide nanoparticles.

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INTRODUCTION

Marine macroalgae have gained significance due to their unique biological properties and high nutritional content (Qiu *et al*., 2022). Among these, *G. corticata* - red seaweed has been utilized for generations as food and fertilizer, and is a rich source of bioactive compounds like minerals, fatty acids and polyphenols (Chellapandian *et al*., 2019). The nanoparticles synthesized with *G. corticata* extract are non- toxic as compared to traditional chemical synthesis and exhibit enhanced functional properties, responsiveness, surface reactivity, and biocompatibility and hence can serve as therapeutic agent (Uzair *et al*., 2020).

Cancer is a chronic disease affected by oxidative stress due to imbalance between Reactive Oxygen Species (ROS) and defensive mechanisms acting against it (Afzal *et al*., 2023). Seaweeds are established sources of natural antioxidants capable of protecting against ROS-induced cell damage (Kumar *et al*., 2021). The IONPs synthesized using *G. corticata* extract can serve as a therapeutic agent in treating cancer. Therefore, it is necessary to evaluate their antioxidant potential to understand the protective function of IONPs against ROS-induced cell damage (Ansari *et al*., 2019). The cytotoxic potential of IONPs has to be determined to assess safety and effectiveness for therapeutic applications. *In vitro* cytotoxic model such as A549 lung cancer provide a reliable platform for initial toxicity assessment (Ayyanaar *et al*., 2019). This preliminary study investigates *G. corticata* - mediated IONPs

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for antioxidant activity and cytotoxic effect on A549 lung cancer cells. This comprehensive strategy emphasizes potential of *G. corticata* as a sustainable source for developing formulations of biologically active nanoparticles with application in lung cancer treatment.

MATERIALS AND METHODS

Collection of *G. corticata*

The samples of *G. corticata* were collected from the Mandapam coast, Rameshwaram, Tamil Nadu, India. The samples were cleaned with distilled water and shade dried for 5-7 days. Samples are powdered and stored for further experimental use (Rosemary *et al.*, 2019).

Nutritional Profiling of *G. corticata*

The nutritional composition of *G. corticata* was determined by standard proximate analysis method with simple modifications (Beshaw *et al.*, 2022). All experiments were performed in triplicated.

Estimation of moisture content

The AOAC (2000) method was used to determine moisture content wherein 5 g algal sample was weighed in a pre-weighed crucible and dried in hot air oven at 105°C until constant weight is achieved and cooled in a desiccator. The samples were reweighed and put back in oven and dried until constant weight achieved. The percent moisture was calculated by equation

$$\% \text{ Moisture} = (w2 - w3) \times 100 / (w2 - w1)$$

w_1 - weight of empty crucible,

w_2 - weight of crucible+sample before drying,

w_3 - Final weight of crucible+sample after drying.

Estimation of Ash Content

2 g of algal sample dried in hot air oven until constant weight achieved. The samples were then transferred to muffle furnace and ashed for 6 hr at 550°C until white powder is formed. The samples are cooled in desiccator and weighed. Percent ash is calculated by equation (Beshaw *et al.*, 2022).

$$\text{Ash } (\%) = (\text{Weight of Ash} / \text{Weight of sample}) \times 100$$

Estimation of Crude protein Content

Lynch & Barbano (1999) method was employed to estimate protein conten. A solution containing 5 g of algal sample, 2 g of Kjeldahl catalyst and 200 mL of sulfuric acid was taken in a digestion flask and boiled until a clear bubble free solution is obtained. Then, 60 mL of water and 6 drops of mixed indicator was added and heated until all nitrogen was distilled. Finally, the obtained solution is titrated against NaOH. Percent crude protein content was calculated using equation.

$$\text{Crude Protein Content } (\%) = ((A-B) \times N \times 14 \times 6.25) / W$$

A - Volume in mL of 0.25N HCl used for titration,

B - Volume in mL of 0.25 N HCl used in Blank titration,

W - Weight of sample in g,

14 - Atomic weight of Nitrogen,

6.25 - Protein - nitrogen conversion factor.

Estimation of Lipid content

To determine lipid content, 5 g of algal sample was taken in Soxhlet and 300 mL of petroleum ether was added and heated 85°C until solvent completely evaporated. The residues were cooled in a desiccator and reweighed (Beshaw *et al.*, 2022). Percent lipid content was calculated by equation.

$$\text{Lipid } (\%) = ((w2 - w1) / w3) \times 100$$

w_1 - weight of empty bottle,

w_2 - weight of bottle+Oil,

w_3 - weight of sample.

Estimation of Fiber content

4 g of algal sample was mixed in 200 mL of Sulfuric acid and boiled for 30 min and filtered. The residue was washed thrice in hot water and 100 mL of 2% of NaOH was added to mixture and boiled for 30 min. Filtered, washed thrice until free from acid. The residue was dried in hot oven air oven at 100°C overnight and weighed. Finally, residue was ashed in muffle furnace at 500°C for 2 hr till grey ash formed and weighed (Beshaw *et al.*, 2022). Percent total crude fiber is calculated by

$$\text{Crude fiber } (\%) = ((w1 - w2) / w3) \times 100$$

w_1 - weight of sample before heating,

w_2 - weight of sample after heating,

w_3 - weight of original sample.

Estimation of Carbohydrate content

The carbohydrate content calculated by equation (Beshaw *et al.*, 2022).

$$\text{Carbohydrate } (\%) = 100 - (\% \text{ Moisture} + \% \text{ Crude Protein Content} + \% \text{ Lipid} + \% \text{ Ash} + \% \text{ Crude fiber})$$

Green Synthesis of IONPs

Aqueous extract of *G. corticata* was used for green synthesis of IONPs. 10 g of powdered seaweed was boiled in 100 mL of

distilled water for 10 min, cooled, and then filtered through filter paper. The filtrate was mixed with 0.1 M ferric chloride solution in 1:1 ratio. The pH was adjusted to 11, and reaction mixture was continuously stirred for 60 min at room temperature before being allowed to settle for an additional 30 min. The resultant colloidal suspension was centrifuged at 10,000 × g for 15 min. The pellet was washed with ethanol, and dried under vacuum and stored at 4 ºC for further analysis (Kiwumulo *et al*., 2022).

Characterization of Nanoparticles

The synthesized IONPs were characterized using UV-visible spectroscopy (Rauf *et al*., 2024), FTIR spectroscopy to identify the functional groups (Minhas *et al*., 2023), and crystallinity by XRD analysis (Kiwumulo *et al*., 2022).

Antioxidant Activity

DPPH Antioxidant Assay

2mL of freshly prepared 100mM DPPH in methanol was added to series of concentrations (10,20, 30, 40, and 50 ug/mL) of synthezised IONPs and incubated for 15 mins in dark at 25 °C followed by recording absorbance at 517 nm (Brand-Williams *et al*., 1995).

ABTS Assay

The ABTS assay was performed with modifications. Briefly, ABTS•$^+$ solution was prepared by oxidizing ABTS (7 mM v/v in Milli-Q water) by 2.45 mM Ammonium Persulfate. The mixture was preincubated overnight in dark and diluted in methanol (1:29). Different concentration of synthesized IONPs were incubated with 2 mL of ABTS•$^+$ reagent at dark for 5 min and the absorbance was measured at 734 nm (Re *et al*., 1999).

FRAP Assay

The FRAP assay was performed by incubating the synthezised IONPs with 1 mL of FRAP reagent (100 mL of 300 mM Acetate buffer (pH 3.6), 10 mL of 10 mM ferric-tripyridyltriazine (in 40 mM HCl), 10 mL of 20 mM $FeCl_3$, and 12 mL Milli-Q water) for a duration of 10 min, followed by the measurements of absorbance at 593 nm (Benzie and Strain, 1996).

Hydrogen Peroxide scavenging Assay

H_2O_2 scavenging activity was determined by incubating IONPs solution with phosphate-buffered H_2O_2 solution (40 mM H_2O_2 in 50 mM phosphate buffer pH 7.4) for 10 min and absorbance measurement at 230 nm (Ruch *et al*., 1989).

Nitric Oxide radical inhibition Assay

For NO scavenging assay, the synthezised IONPs were incubated in 1.5 mL of 10mM sodium nitroprusside in PBS for 30 min and thereafter, addition of 1.5 mL of Griess reagent and immediately absorbance was read at 540 nm (Green *et al*., 1982; Narasimhan *et al*., 2013).

Cytotoxicity Studies

The cytotoxic effect of IONPs was evaluated on A549 human lung cancer cell lines. The cells were revived and cultured in DMEM supplemented with 10% FBS and 1% streptomycin. The cells were maintained at 37 ºC with 5% CO_2. Once confluency was achieved, cells were trypsinized and a total of 5 × 10^4 cells per well were seeded into 96-well plates and cultured overnight. The cells were treated with increasing concentrations (20 - 120 µg/mL) of the synthesized IONPs and incubated for 24 hr. The cells were treated with MTT solution (5 mg/mL), 10 µL into each well and incubated for 4 hr. 100 µL of MTT Dissolving Solution (DMSO) was dispensed into each well to dissolve formazan and absorbance was measured at 570 nm (Narasimhan *et al*., 2013).

RESULTS

Nutritional Profiling of *G. corticata*

The proximate composition analysis of *G. corticata* (Table 1) revealed a moisture content of 9.52 ± 2.7%, confirming its water-retentive capacity and implications for storage stability and processing. The ash fraction was measured at 13.76 ± 1.7%, reflecting the substantial mineral load typical of marine macroalgae. Carbohydrates constituted the major component at 57.40 ± 0.6%, underscoring its potential as a rich dietary energy source. Protein content was 13.39 ± 0.9%, while lipids accounted for 2.75 ± 1.1%, indicating moderate nutritional value. Additionally, a secondary ash fraction was recorded at 3.18 ± 1.8%, which may correspond to insoluble mineral residues.

Synthesis and Characterization of IONPs

The successful green synthesis of IONPs was visually evident by a color shift from yellowish-brown to deep brown, indicating reduction of ferric ions. UV-visible spectroscopy displayed a sharp absorption peak at 294 nm, signifying the surface plasmon resonance typically associated with Fe_3O_4 nanoparticles (Figure 1A). FTIR spectroscopy revealed prominent bands indicative of hydroxyl, carbonyl, and alkene functional groups, confirming the involvement of polysaccharides and phenolics from the seaweed extract in both reduction and capping (Figure 1B). The XRD analysis of green-synthesized iron oxide nanoparticles as shown

Table 1: Nutritional analysis of *G. corticata*. Values are articulated as Mean±S.D (*n*=3).

Sl. No.	Content	Percentage (%)
1	Moisture	9.52±2.7
2	Ash	13.76±1.7
3	Carbohydrate	57.40±0.6
4	Protein	13.39±0.9
5	Lipid	2.75±1.1
6	Fiber	3.18±1.8

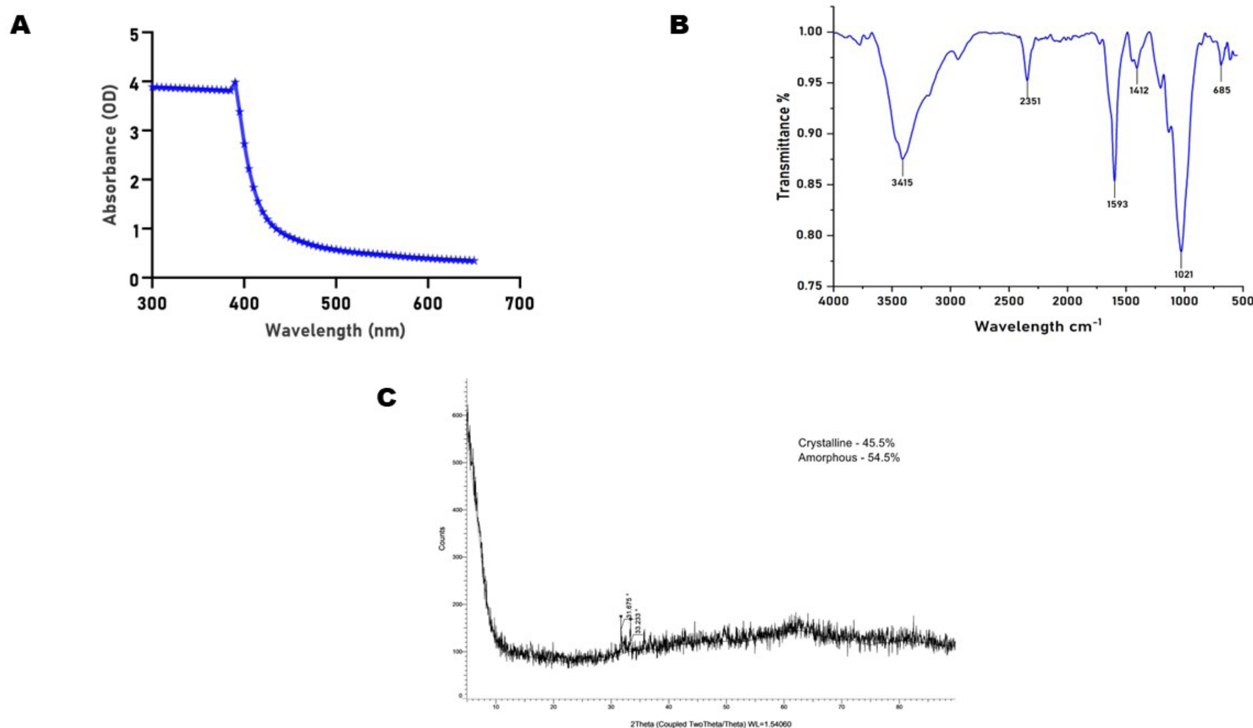

Figure 1: Characterization of *G. corticata*-mediated IONPs. A) UV–Vis spectroscopy, B) FTIR analysis, C) XRD pattern indicating amorphous nature.

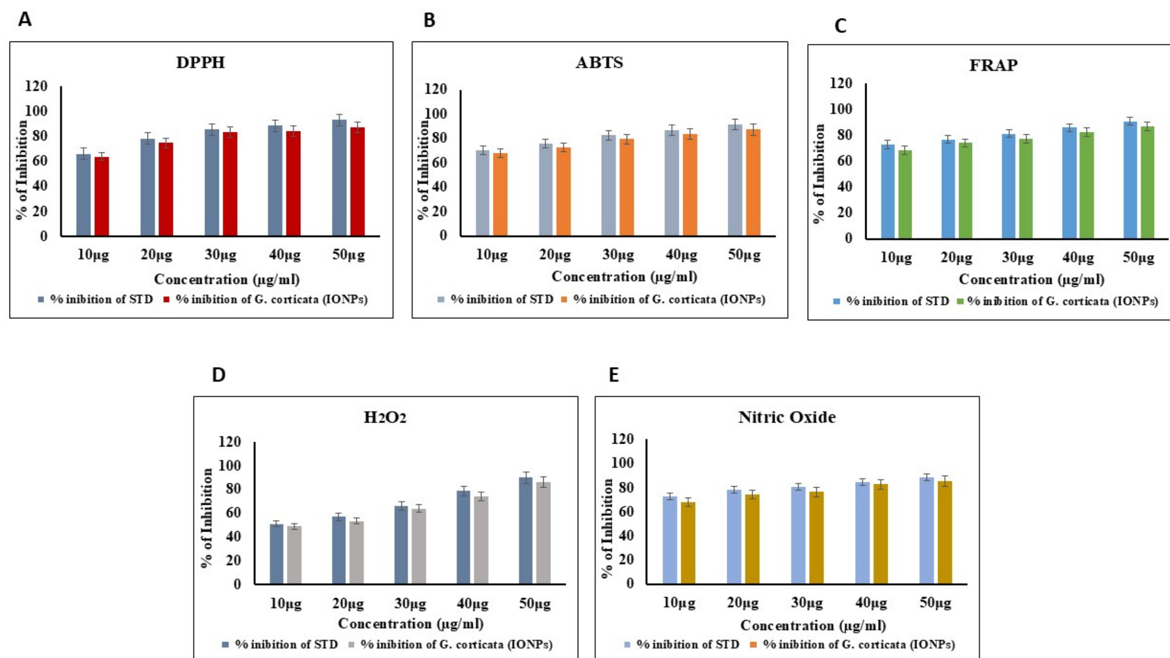

Figure 2: Antioxidant potential of *G. croticata*-mediated IONPs. A) DPPH, B) ABTS, C) FRAP, D) Hydrogen peroxide, and E) Nitric oxide inhibition assays.

in Figure 1C revealed crystalline of 45.5 % and amorphous of 54.5 %.

Antioxidant Activity

The antioxidant efficacy of *G. corticata* -mediated IONPs was validated through multiple assays. DPPH radical scavenging (Figure 2A) increased from 63.84% to 87.16% (for 10 μg/mL and 50 μg/mL, respectively). Similar concentration-dependent increase in antioxidant activity was observed in ABTS (67.94% to 87.12%), FRAP (68.53% to 87.11%), H_2O_2 (47.1% to 87.4%), and nitric oxide scavenging (68.21% to 85.62%) assays (Figures 2B-E, respectively). These findings suggest a high radical quenching

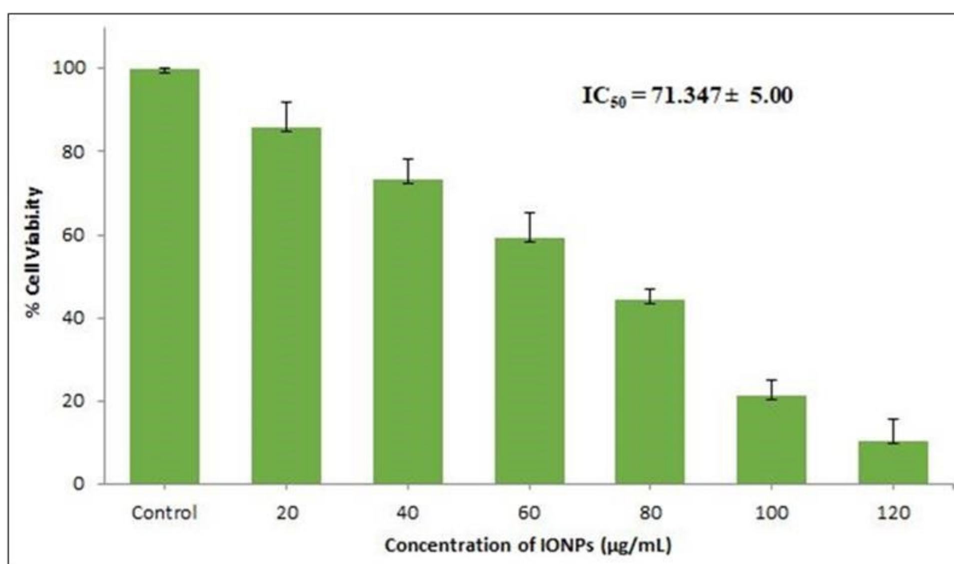

Figure 3: The cytotoxic effects of *G. corticata* derived IONPs on A549 human lung cancer cells.

capacity, which can be attributed to the combined impacts of bioactive compounds of seaweed extract and surface reactivity of IONPs.

Cytotoxicity Studies

The cytotoxic potential of IONPs synthesized from *G. corticata* was evaluated against A549 lung cancer cells. The results demonstrated a dose-dependent decrease in cell viability following treatment with increasing concentrations of IONPs (20–120 µg/mL) (Figure 3).

Compared with the control group, which showed nearly 100% cell viability, treatment with 20 µg/mL resulted in approximately 86% viability, which gradually decreased to 73% at 40 µg/mL and 59% at 60 µg/mL. A more pronounced cytotoxic effect was observed at higher concentrations, with cell viability reduced to 45% at 80 µg/mL, 22% at 100 µg/mL, and around 10% at 120 µg/mL.

The half-maximal inhibitory concentration (IC_{50}) of the synthesized IONPs against A549 cells was calculated to be 71.347 ± 5.00 µg/mL, indicating a significant inhibitory effect on lung cancer cell proliferation. These findings suggest that *G. corticata*-mediated IONPs exhibit concentration-dependent cytotoxic activity against A549 lung cancer cells.

DISCUSSION

The proximate analysis of *G. corticata* show abundant carbohydrates and other nutrient content emphasizing its rich source of macronutrients. This finding corroborates the work of Rosemary *et al*., (2019), who emphasized red seaweeds as mineral-rich dietary components.

The IONPs synthesized by *G. corticata* extract shows no toxicity and are environmentally safe as compared to traditionally synthesized nanoparticles. The IONPs exhibit typical spectral features consistent with findings by Anbarasan and Arivalagan (2024), who reported similar UV-vis absorption peaks in plant extract-mediated iron oxide nanoparticle synthesis. FTIR pattern is consistent with the results of Minhas *et al*., (2023), where marine polysaccharides facilitated nanoparticle stabilization. The XRD analysis show high amorphous content suggesting partial crystallization influenced by the phytochemicals in the plant extract, consistent with studies of (Yadav *et al*., 2020).

IONPs exhibit strong antioxidant activity and similar results were reported by Rajivgandhi *et al*., (2020), who showed that seaweed-derived nanoparticles enhanced radical scavenging due to their high surface area and synergistic interactions with phenolic constituents. (Long *et al*., (2022) also highlighted the capacity of brown seaweed-based nanoparticles to eliminate oxidative stress *in vivo*. Our results reinforce these findings and confirm that red seaweed-based IONPs possess considerable potential in antioxidant therapy and nutraceutical formulations.

The cytotoxic effect of IONPs on A549 lung cancer cells provides a way for development of anti -cancer therapy, though further experimentation is necessary. These findings are consistent with those of Mohanta *et al*., (2022), who reported significant cytotoxic effects of seaweed-based iron nanoparticles on various cancer cell lines, attributing their activity to oxidative damage and mitochondrial dysfunction.

CONCLUSION

This study successfully demonstrated the green synthesis of IONPs using the red seaweed *G. corticata*, highlighting its potential as a sustainable bioresource. Nutritional profiling confirmed the richness of seaweed in essential macronutrients and minerals, supporting its use in functional food and biomedical applications. The synthesized nanoparticles exhibited strong, dose-dependent antioxidant activity across multiple assays and moderate cytotoxicity toward A549 lung cancer cells, indicating promising

therapeutic potential. These findings align with existing literature and emphasize the dual role of *G. corticata* as a candidate in both nutraceuticals and nanomedicine. Future work should focus on detailed mechanistic studies and *in vivo* evaluations to further explore the clinical relevance and safety of these nanoparticles. Overall, *G. corticata*-mediated iron oxide nanoparticles present a valuable platform for developing eco-friendly and effective antioxidant, antimicrobial, and anticancer agents.

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ABBREVAITIONS

IONPs: Iron Oxide Nanoparticles; ***G. corticata*:** *Gracilaria corticata*; **FTIR:** Fourier-Transform Infrared Spectroscopy; **XRD:** X-ray Diffraction; **DPPH:** 2,2-Diphenyl-1-Picrylhydrazyl; **ABTS:** 2,2′-Azino-Bis(3-Ethylbenzothiazoline-6-Sulfonic Acid); **FRAP:** Ferric Reducing Antioxidant Power; **ROS:** Reactive Oxygen Species; **H_2O_2:** Hydrogen Peroxide; **IC_{50}:** Half Maximal Inhibitory Concentration; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; **DMEM:** Dulbecco's Modified Eagle Medium; **DMSO:** Dimethyl Sulfoxide; **AOAC:** Association of Official Analytical Chemists; **PBS:** Phosphate-Buffered Saline.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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