International Conference on Sustainable Biotechnology (ICoSBi)
Available on-line at: https://sicash.sliit.lk/

Phytochemical Profiling and Bioefficacy of Macroalgae Extracts Enhanced with ZnO Nanocomposites

T. Mandawala[1], N. Hettiarachchi[1], M. Jayanetti[1], C. Thambiliyagodage*[1]

[1]*Department of Applied Sciences, Faculty of Humanities and Sciences, Sri Lanka Institute of Information Technology, SLIIT-Malabe Campus, Malabe, 10115, Sri Lanka.*

Corresponding author*: charitha.t@sliit.lk

Abstract

Combining marine macroalgae with metal nanoparticles presents a viable way to increase the therapeutic potential of these valuable natural bioactive chemicals. This study aimed to investigate the potential of Zinc oxide (ZnO) nanoparticles synthesized using marine macroalgae to enhance the antimicrobial, antioxidant and anti-inflammatory activities of the algal extracts, and to compare these effects across different algal species. Methanolic extracts from macroalgae *Gelidium* sp. (SB1), *Sargassum ilicifolium* (SB2), *Turbinaria ornata* (SB3), and *Cladophora* sp. (SB4) collected from marine habitats from Sri Lanka and their ZnO composites (named as SB1+ ZnO accordingly) were evaluated for antioxidant (DPPH and Folin–Ciocalteu Assay), anti-inflammatory, and antibacterial (using agar well method and MIC/MBC assay) properties. Glycosides, steroids, and flavonoids, in the algae samples were identified by phytochemical screening. Phenols and tannins were only presented in SB2 and SB4. The ZnO composite of SB2 exhibited the highest phenolic content (0.488 mg GAE/g), which was elevated to 0.604 mg GAE/g in SB4+ ZnO. In SB2+ZnO, a 33% increment in Anti-inflammatory activity was observed at 1600 µg/mL, and antioxidant activity was enhanced in SB2+ZnO (IC_{50} ≈230 µg/mL) which was at 672 µg/mL in SB2. With MICs ranging from 20 to 40 µg/mL, ZnO and ZnO–algae composites demonstrated antibacterial efficacy against *S. aureus*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae* (zones: 10–14.8 mm). Given the improved bioactivities, our results demonstrate the potential of *S. ilicifolium* and *T. ornata* as well as their ZnO composites, to be used in cosmeceutical and pharmaceutical industries.

***Keywords*:** Antioxidant activity, anti-inflammatory activity, antimicrobial activity, macroalgae, ZnO nanoparticles

https://doi.org/10.54389/CPZN2734

Introduction

Seaweeds, also referred to as marine macroalgae, are multicellular, aquatic photosynthetic organisms that are widely distributed in marine habitats, especially along coastlines. Green (*Chlorophyta*), brown (*Phaeophyta*), and red (*Rhodophyta*) algae are categorized according to their pigment content, which dictates their biological range and light absorption at varying ocean depths. Red algae are known for their agar and carrageenan, brown algae for their laminarin, alginate, and fucoidan, and green algae for their starch and ulvan (Lomartire & Menaa, 2022). These substances have antioxidants, antibacterial, and anti-inflammatory properties in addition to supporting the structural integrity of algae and being crucial to their bioactivity. Macroalgae are being studied extensively for use in cosmetics, nutraceuticals, and pharmaceuticals because of their diverse phytochemical properties. For instance, polyphenols such as phlorotannin with potent antibacterial and antioxidant properties, are especially abundant in brown algae (Generalić Mekinić et al., 2019). It is widely known that zinc oxide nanoparticles (ZnO NPs) have broad-spectrum antibacterial activity, can produce reactive oxygen species (ROS), and can be used as antioxidants and UV filters (Pasquet et al., 2014). ZnO NPs provide an economical and environmentally friendly alternatives to biologically active compounds when they are produced utilizing green techniques with plant or algae extracts. When ZnO NPs and macroalgal extracts are combined, their bioactivities may be enhanced synergistically. However, the majority of macroalgae found in coastal ecosystems in Sri Lanka are still not well understood, despite growing attention worldwide. Furthermore, not many studies use standardized techniques to provide a comparison of data across various algal species. To fill these research gaps, this study investigated the phytochemical profile and antibacterial, anti-inflammatory, and antioxidant bioactivities of ZnO nano composites made from four marine macroalgae found in Sri Lanka: *Gelidium* sp., *Sargassum ilicifolium*, *Turbinaria ornata*, and *Cladophora* sp. These species were chosen for their high phytochemical and antioxidant activity and their widespread, abundant presence in Sri Lankan coastal waters.

Materials and Methods

Green Synthesis of ZnO Nanoparticles

NaOH solution was added to the zinc acetate dihydrate solution dropwise to obtain a white precipitate and stirring was continued overnight. The precipitate was washed with distilled water until a neutral pH was reached, followed by drying at 80 °C. After being gathered from Hikkaduwa beach in Sri Lanka, the macroalgae: Red algae- *Gelidium* sp. (SB1), brown algae- *Sargassum ilicifolium* (SB2), *Turbinaria ornata* (SB3) and green algae-*Cladophora sp*. (SB4) were washed with distilled water. The extracts were taken by sonication at 35 °C. The extracts obtained were centrifuged to remove any debris. Pure extract was mixed with the ZnO nanoparticles, and stirring continued for 24 hours at room temperature. The temperature was then increased to 50 °C, and stirring continued until methanol completely evaporated. SEM was used to examine the morphology, size, shape and agglomeration of the ZnO–macroalgae composites

Phytochemical screening

Each methanolic extract was subjected to standard qualitative tests to identify proteins (Millon's and Ninhydrin tests), carbohydrates (Fehling's, Benedict's, and Iodine tests), phenols/tannins ($FeCl_3$ test), glycosides (Liebermann's, Salkowski, Keller–Kilani tests), flavonoids (Shinoda test), and saponins (foam test). For every test, the results were noted as either "positive" or "negative".

Antioxidant activity of marine algae

A. Folin–Ciocalteu Assay

Each ZnO–algae composite and algal extract was produced in DMSO as a stock solution (1 mg/mL), which was then serially diluted (10–50 μg/mL). After adding 0.1 mL of 0.5N Folin-Ciocalteu reagent to each 0.5 mL sample, the mixture was left to incubate for 15 minutes in the dark. After adding 2.5 mL of 7% sodium carbonate, the mixture was incubated at 35 °C for two hours. At 760 nm, absorbance was measured. A gallic acid calibration curve (Y = 0.0037x + 0.1102; R2 = 0.994) was used to quantify the total phenolic content (TPC), which was then expressed as mg GAE/g dry algae. Every test was conducted in triplicate.

B. DPPH Radical Scavenging Assay

A solution was prepared by dissolving 3.94 mg DPPH in 100 mL of methanol and kept at room temperature (27°C) in the dark for one hour. From a 1 mg/ml stock, test concentrations (10–50 μg/ml) were made. 500 μl of DPPH solution was combined with 3 ml of each test sample, and the mixture was left to incubate for 30 minutes in the dark. Absorbance was measured at 517nm. (Meiyasa et al,2024). Scavenging effect (%) was calculated using:

A_c- absorbance of the control

Scavenging effect (%) = $[(A_c - A_s) / A_c] \times 100$,

A_s- absorbance of the sample

IC50 values were derived from linear regression equations, and the Antioxidant Activity Index (AAI) was calculated as: AAI = IC50(sample) / IC50(DPPH).

Anti-inflammatory Activity

Egg albumin was used to measure the prevention of protein denaturation. In 2 mL of methanol, sample concentrations ranging from 100 to 1600 μg/mL were generated. 200 μL of 1% egg albumin and 2.8 mL of PBS (pH 6.4) were combined with each. After 30 minutes of incubation at 37 °C, the mixtures were heated for 15 minutes at 70 °C. Absorbance was measured at 660 nm. The following was used to determine the Percentage inhibition: (Shunmugaperumal, 2016)

Percentage inhibition = $[A_s / A_c) - 1] \times 100$

A_s- absorbance of the sample

A_c- absorbance of the control

Antibacterial Activity

Antibacterial activity against *S. aureus, K. pneumoniae, P. aeruginosa*, and *E. coli* was assessed. Bacterial suspensions were made according to the 0.5 McFarland standard (5×10^5 CFU/ml). ZnO and ZnO–algae composites from Batch 1 and methanol extracts from Batch 2 were evaluated. The extracts (10, 20, 40 mg) were diluted in 1 ml DMSO and then sonicated. Each sample was added to inoculated Mueller-Hinton Agar (MHA) plates with 70 μl using the agar well diffusion method, and the plates were incubated at 37 °C for 18 hours. Amoxicillin was used as the positive control and DMSO/water as the negative control when measuring the zones of inhibition. Every test was conducted in triplicate.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

ZnO composites were serially diluted in DMSO at concentrations ranging from 5 to 160 mg/mL. A 500 μL bacterial culture (5 × 105 CFU/mL) was used to inoculate each. To calculate the MIC, turbidity was measured following 18 to 24 hours of incubation at 37 °C. 2 μL from each non-turbid tube was spread out

on MHA plates and allowed to incubate. The lowest concentration that did not exhibit colony growth was referred to as MBC. Every one of the four bacterial species was tested.

Results and Discussion

SEM Imaging of ZnO with algae extracts

The SEM images of the ZnO functionalized with the different Algae extracts are shown in Figure 1. In all four ZnO–macroalgae composites, the morphology of the ZnO shows agglomeration of the irregular shaped materials. Further, with functionalization of the ZnO, the surface patterns differ from each other as the Algal species used for the functionalization is different (Alprol,2024).

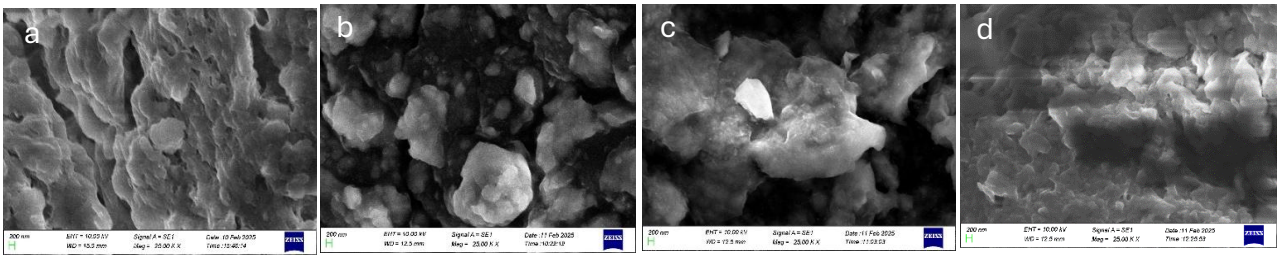

Figure 1: *a -SB1_ZnO, b-SB_ZnO, c-SB3_ZnO, d-SB4_ZnO.*

Phytochemical Screening

Glycosides, steroids, and flavonoids were present in every extract. Only SB1 and SB2 had terpenoids, whereas SB2 and SB4 contained phenols and tannins. There were no proteins or saponins found. These results indicate that flavonoids and terpenoids have inherent bioactivity and represent characteristics of typical algae groups.

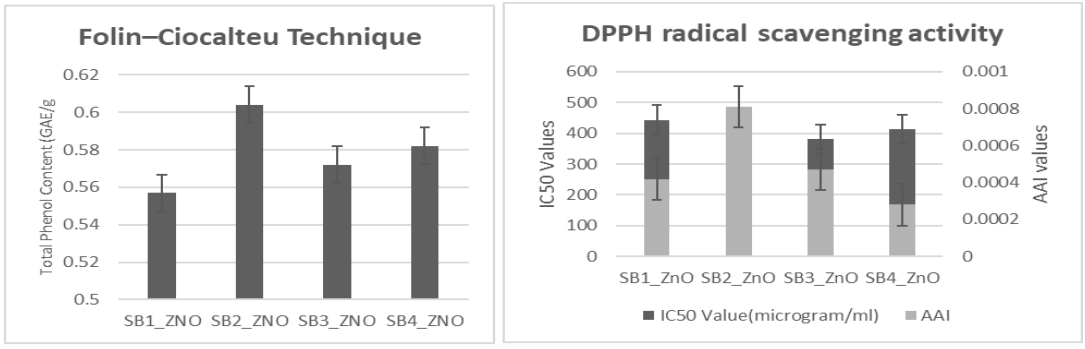

Figure 2: *Total Phenolic Content (TPC) in the ZnO combined with algae extracts.*

Antioxidant activity

A. Folin-Ciocalteu Assay

SB2 has the highest phenolic concentration (0.488 g GAE/mg). All samples' phenolic levels increased when combined with ZnO; SB2+ZnO: 0.604 g GAE/mg; SB4+ZnO: 0.582 g GAE/mg; SB3+ZnO: 0.572 g GAE/mg; SB1+ZnO: 0.557 g GAE/mg as indicated in Figure 2. This improvement implies that ZnO enhances the antioxidant activity of algae extracts. SB2 had the highest phenolic content, which is consistent with the fact that brown sargassum is known to produce high-molecular-weight phenolics. The species with greater phenolics (SB2, SB3) demonstrated increased radical scavenging in our data, demonstrating the well-established relationship between phenolics and antioxidant activity (Deyab, 2016). Generalić et al. (2019), reported that brown algae are rich in phenolic compounds.

B. DPPH Radical Scavenging Assay

SB2 and its ZnO nanocomposite (SB2+ZnO) showed the greatest antioxidant activity among the investigated samples, as indicated by the lowest IC_{50} values as indicated in Figure 3. When ZnO was introduced, the antioxidant capacity remarkably increased, suggesting a synergistic effect that was probably caused by improved electron donation and reactive oxygen species neutralization from the combined action of ZnO nanoparticles and the phenolic compounds found in it. These findings are in line with earlier research that highlights the high concentration of phlorotannin in brown algae, which are strong antioxidants (Meiyasa et al,2024).

Anti-inflammatory Activity

In a concentration-dependent way, all combined samples prevented the denaturation of egg albumin as indicated in Figure 4. The most efficient combination was observed in SB2+ZnO, at 1600 µg/mL which produced ~50% inhibition, while SB2 alone produced ~16.7%. Inhibition was minor or negative at lower doses. ZnO extract composites outperformed compared to the extracts alone. These patterns are consistent with claims that ZnO NPs have an NSAID-like ability to suppress protein denaturation. In a Study by Varghese et al. (2024) ZnO NPs demonstrated dose-dependent suppression of albumin denaturation which verified our findings. The activity is possibly the result of algal pigments, phlorotannin and sulfated polysaccharides working together with ZnO (Wijesinghe, 2012).

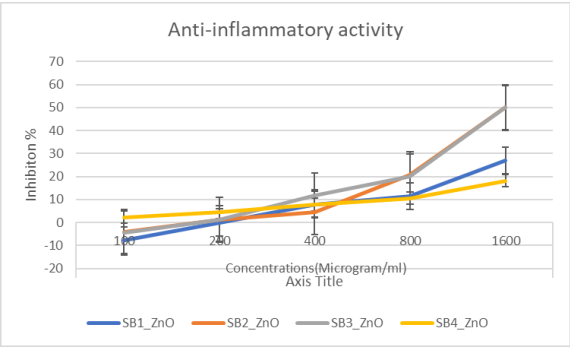

Figure 3: *Anti-inflammatory activity of the combined extracts*

Anti-bacterial Activity

Both Gram-positive (*S. aureus*) and Gram-negative (*K. pneumoniae, E. coli, and P. aeruginosa*) bacteria were susceptible to the antibacterial activity of ZnO nanoparticles, methanolic extracts, and ZnO-algae composites (Figure 5). The greatest inhibition was seen against *E. coli* (14.83 ± 1.07 mm, ZnO) and S. aureus (14.5 ± 0.5 mm, SB1+ZnO). Strong activity against *K. pneumoniae* was demonstrated by SB3 and SB4 composites up to 14.33 ± 1.69 mm, and this activity increased in higher ZnO composite concentration. On *P. aeruginosa*, SB1+ZnO (20 mg/ml) had the greatest effect compared to methanol extracts alone.

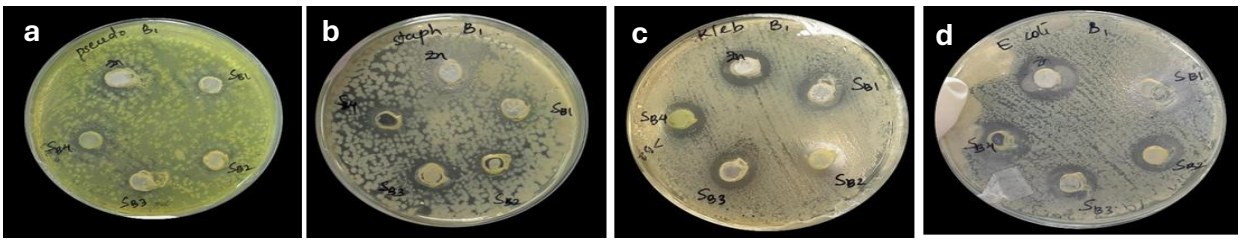

Figure 4*: Antibacterial activity of ZnO nanoparticles synthesized using different macroalgal species (SB1–SB4) at 40 mg/mL against four bacterial strains using the well diffusion method: (a) Pseudomonas aeruginosa, (b) Staphylococcus aureus, (c) Klebsiella pneumoniae, and (d) Escherichia coli. Clear zones of inhibition indicate effective antimicrobial activity.*

ZnO primarily inhibits bacteria by producing Zn^{2+} ions, which cause cell death by interfering with enzyme activity, DNA replication, and redox balance. Additionally, it produces reactive oxygen species (ROS) that harm biological components, such as hydrogen peroxide and hydroxyl radicals. Antibacterial activity is increased by smaller nanoparticles because they improve membrane contact and penetration. By enhancing dispersibility and regulating ion/ROS release, surface changes can further maximize ZnO's activity (Jiang, 2009).

MIC and MBC assay

Table 1*: MIC values, MBC values and MIC/MBC ratios of the combined Nanomaterial*

Species	**SB1_ZnO**		**SB2_ZnO**		**SB3_ZnO**		**SB4_ZnO**		**ZnO**		MBC/MIC ratio				
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	**SB1_ZnO**	**SB2_ZnO**	**SB3_ZnO**	**SB4_ZnO**	**ZnO**
E.coli	40	40	40	80	40	40	20	80	10	10	1	2	1	4	1
Staph	20	80	40	160	40	80	40	80	10	20	4	4	2	2	2
Pseudo	40	160	40	80	40	80	40	160	20	40	4	2	2	4	2
Kleb	40	80	40	160	40	80	40	160	40	40	2	4	2	4	1

The bactericidal activity of all ZnO–macroalgae composites against *E. coli, P. aeruginosa, K. pneumoniae*, and *S. aureus* was validated by MIC and MBC assays. MBC was the lowest concentration at which no colonies formed during subculturing, whereas MIC, which was determined by the lack of turbidity, indicated the lowest concentration necessary to prevent visible bacterial growth. As seen in Table 1 MBC/MIC ratios ≤ 4

were demonstrated by all ZnO- algae composites, indicating a bactericidal mechanism of action. The natural color of the ZnO-algae composites often made it difficult to see clearly, even if turbidity reduction in MIC tubes indicated bacterial suppression. Subculturing was therefore required to precisely verify total bacterial eradication. These results are consistent with ZnO's well-established processes, which include the production of ROS and the release of Zn^{2+} ions to effectively kill microorganisms (Jyothsna,2023).

Conclusion

When coupled with ZnO nanoparticles, brown algae *Sargassum ilicifolium* and *Turbinaria ornata* showed the most significant bioactivity among the examined species, indicating their higher therapeutic potential. This study adds to the growing evidence that brown macroalgae are among the most promising options for creating bioactive formulations based on nanomaterials. These results highlight the importance of brown algae as a sustainable resource for upcoming cosmeceutical pharmaceutical and biological uses, especially considering their abundance in Sri Lankan marine habitats.

References

Alprol, A. E., Eleryan, A., Abouelwafa, A., & El-Ashram, S. (2024). Green synthesis of zinc oxide nanoparticles using *Padina pavonica* extract for efficient photocatalytic removal of methylene blue. *Scientific Reports*, *14*, 32160. https://doi.org/10.1038/s41598-024-80757-9

Deyab, M., Elkatony, T., & Ward, F. (2016). Qualitative and quantitative analysis of phytochemical studies on brown seaweed, *Dictyota dichotoma*. *International Journal of Engineering Development and Research, 4(2)*, 674–678.

Generalić Mekinić, I., Skroza, D., Šimat, V., & Smole Možina, S. (2019). Phenolic content of brown algae (*Pheophyceae*) species: Extraction, identification, and quantification. *Biomolecules, 9(6),* 244. https://doi.org/10.3390/biom9060244

Jiang, W., Mashayekhi, H., & Xing, B. (2009). Bacterial toxicity comparison between nano- and micro-scaled oxide particles. *Environmental Pollution*, *157(5)*, 1619–1625. https://doi.org/10.1016/j.envpol.2008.12.025

Jyothsna, S., Lavanya, T., Azeez, M. A., Naresh, K., & Thangaraju, K. (2023). Functionalized nanomaterials with enhanced anti-microbial activity. In A. A. Shanmugam, A. M. Al-Mayouf, & M. M. Rahman (Eds.), *Antimicrobial nanosystems* (pp. 255–293).

Lomartire, S., & Menaa, F. (2022). An overview of potential seaweed-derived bioactive compounds and their applications. Marine Drugs, 20(2), 141. https://doi.org/10.3390/md20020141

Meiyasa, A. I., Aji, M. P., Setyaningrum, D. L., & Wijayanti, H. S. (2024). Phytochemical analysis and antimicrobial activity of *Sargassum cristaefolium*. *Food Research, 8(1)*, 82–91. https://doi.org/10.26656/fr.2017.8(1).050

Pasquet, J., Chevalier, Y., Pelletier, J., Couval, E., Bouvier, D., & Bolzinger, M. A. (2014). The contribution of zinc ions to the antimicrobial activity of zinc oxide. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 457*, 263–274.

Shunmugaperumal, T., & Kaur, V. (2016). In vitro anti-inflammatory and antimicrobial activities of azithromycin after loaded in chitosan- and Tween 20-based oil-in-water macroemulsion for acne management. *AAPS PharmSciTech, 17,* 700–709. https://doi.org/10.1208/s12249-015-0401-2

Wijesinghe, W. A. J. P., & Jeon, Y. J. (2012). Enzyme-assisted extraction (EAE) of bioactive components: A useful approach for recovery of industrially important metabolites from seaweeds: *A review. Fitoterapia, 83(1),* 6–12. https://doi.org/10.1016/j.fitote.2011.10.016