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DIFFERENT ANTIOXIDANT PROPERTIES FROM PADINA GYMNOSPORA MARINE ALGAE MEDIATED COPPER NANOPARTICLES

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Abstract:

Introduction :

Padina gymnospora are a kind of marine brown algae that are well-known for their many bioactive components, including antioxidants. Our bodies produce free radicals on a regular basis, and their buildup can cause serious health issues and, ultimately, death. Numerous ailments, including heart attacks, are at a lower risk thanks to antioxidants. Because they are less volatile and more stable at high temperatures than synthetic antioxidants, more natural antioxidants are preferable. The inclusion of copper nanoparticles improves the antioxidant qualities that are already present.

Aim:

To synthesise Padina gymnospora mediated copper nanoparticles and to hence study their antioxidant properties.

Materials and methods :

Padina gymnospora samples are collected, cleaned with distilled water, dried, and powdered. The manufacturing of methanol extract begins with the powdering of crude oil extract, which is subsequently dried for a few hours. To identify the bioactive components from crude extract, further antioxidant assays (DPPH, Nitric oxide assay, SEM, EDX , light absorbance) were conducted.

Results:

In tests using the DPPH and nitric oxide assays, it was shown that the seaweed Padina gymnospora's antioxidant ability increased with larger extract concentrations.

Conclusion:

In comparison to other earlier investigations, this study found that the ethanol extract of seaweed exhibited stronger antioxidant properties. Thus proving the antioxidant potential of Padina gymnospora as a more biotic alternative of an efficient antioxidant with enhanced properties exhibited by incorporating it in copper nanoparticles.

Keywords: Padina Gymnospora-Marine algae-Copper nanoparticles-Antioxidant properties-Free radical scavenging-DPPH (2,2-diphenyl-1-picrylhydrazyl) assay- Nitric oxide assay- ethanolic crude extract- biotic

Introduction:

In recent years, there has been increasing interest in marine organic entities as a viable and promising source for marine life.Due to its extraordinary flexibility, rapid development, and resource sustainability, seaweed has become one of the most promising resources. It is a significant development that will help to prevent future resource shortages. Due to the rising production and demand for seaweed globally over the past several years, algal resources have reached a high stage of growth(1).According to the Food and Agriculture Organization (FAO) data, the global seaweed output (both aquaculture and wild) has increased nearly three-fold from 118,000 tons to 358,200 tons from 2000 to 2019.(FAO 2021).Seaweeds provide a variety of medical benefits, including pharmacological and anti-adhesive properties due to their antibacterial and antinociceptive properties(2,3)(4).They are one of the commercially important marine renewable resources and are the natural repository of different vitamins, minerals, trace elements, proteins, iodine, bromine and bioactive substances.Brown seaweeds are aquatic photosynthetic algae that are members of the kingdom Chromista, class Phaeophyta, and domain Eukarya(5).The planet is home to 1500 different types of brown seaweed. As a traditional herbal remedy, seaweed has been used particularly in Asian nations to treat gastrointestinal issues, cough, boils, ulcers, asthma, cough, and headaches as well as vegetables. Recent studies have shown that dietary seaweeds contain a high concentration of functionally bioactive compounds like carotenoids, polysaccharides, polyphenols, and sterols, which may have antioxidant properties in addition to antimicrobial, anticoagulant, anti-inflammatory, and antiviral properties for various diseases. Additionally, dietary seaweeds are a good source of carbohydrates, dietary fibre, proteins and peptides, vitamins, minerals, and fats.The thick thallus, the movement of sporangia mostly on the dominant surface of the thallus, and the ephemeral indusium are characteristics of Padina gymnospora. A significant amount of carbohydrates, fatty proteins, and other minerals are also included in Padina Gymnospora's nutritional profile, along with elements that support cell growth and other vitamins (C and E).

So far antibiotics and various other synthetic drugs have been in use for treatment of various ailments.But antibiotics effectiveness is now at danger due to the alarming growth and spread of "multi-drug-resistant (MDR) bacteria" that pose a severe threat to community health throughout the biosphere. Antibiotic therapy has failed in hundreds of cases, and antibiotic resistance is becoming increasingly common in many bacterial diseases.Nanomaterials have unavoidably being used in recent years due to their amazing optical, physical, chemical, and mechanical capabilities.Metallic nanoparticles are a new class of materials with applications in medicine, pharmaceutical and agriculture. Using biological, chemical and physical approaches, nanoparticles with amazing properties are obtained.Copper is one of the most abundant elements found on Earth. It has played an important part in history, given its many properties, like good electrical and thermal conductivity, high corrosion resistance and increased malleability. It has been used in ornaments, weapons and coins since the early 14th century.(5,6). Due to their numerous pharmacological uses, CuO-NPs have captured researchers' attention(7).Copper oxide nanoparticles are also gaining popularity on behalf of their cheap and abundance in contrast to gold and silver metals and also their capable potential application in many fields.

Antioxidants are groups of compounds that neutralise free radicals and reactive oxygen species (ROS) in the cell(7,8).Copper nanoparticles (CuNPs) have also sparked interest in the field of antioxidants due to their unique properties and potential health benefits. These nanoparticles, composed of copper atoms, exhibit distinct physicochemical attributes that contribute to their antioxidant activity. Research suggests that CuNPs can scavenge free radicals and reactive oxygen species (ROS), which are associated with oxidative stress and various diseases. Their small size and high surface area-to-volume ratio enhance their ability to interact with biological molecules, making them potential candidates for antioxidant therapy. Antioxidants are important as they provide protection against damage caused by free radicals and play important roles in the development of many chronic diseases including cardiovascular diseases, ageing, heart disease, anemia, cancer, inflammation. Also a large portion of the reducing agent is found in seaweed which transforms the metal salts into the matching metal nanoparticles (MNPs) without generating any negative side effects(9)

The objective of the current study is to synthesise Padina gymnospora mediated copper nanoparticles, and the further evaluation of its antioxidant potential using DPPH and nitric oxide assay , confirming the nature of the particle and its purity and to further conclude if Padina gymnospora mediated copper nanoparticle can prove to be a more potential biotic alternative to the non biotic antioxidants.

Materials and methods:

The present study was undertaken by Saveetha dental college and hospitals, Chennai with the study being conducted over a period of three months , before the results and conclusion were plotted.

Sample collection and preparation

The fresh seaweed sample Padina gymnospora was collected from Gulf of Mannar biosphere reserve , Tamil Nadu. The samples were washed thoroughly with tap water then shade dried on table tissue paper for 4 weeks and ground into fine powder using mortar and pestle.

Preparation of extract

10g of dried powdered seaweed sample was mixed with 100ml of ethanolic extract and incubated for 24 hours at ambient temperature. Then the mixture was filtered through whatman filter paper (No.4), the filtrate was centrifuged at 3000 rpm for 10 mins , followed by filtration through 0.45 micrometre syringe micro filter.At last, the solvent was evaporated via a vacuum rotary evaporator to obtain dried powder. Then the samples were stored in an aluminium container at 4 degree centigrade for further analysis.

Total antioxidant activity

The total antioxidant activity of the crude seaweed was determined by the following method; 0.3ml of sample was prepared in different concentrations with 3ml of reagent solution - 0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate. Reaction mixture was incubated at 95 degree centigrade for 90 minutes in a water bath: Absorbance of all sample mixtures was measured at 579nm.

DPPH Assay

The antioxidant potential of seaweed extract was determined on the basis of their scavenging activity of the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical. Different concentrations (0.5-3mg/ml) of samples were mixed with 2.9ml diphenylpicrylhydrazyl (DPPH) solution (120 M) in ethanol and incubated in darkness at 37°C for 30 minutes.The absorbance was recorded at 579 nm.

Inhibition of free radical by DPPH in percentage (I %) was calculated with the following equation:

Percentage of Inhibition (I %) = (A blank - A sample)/A blank x 100.

Where, A blank is the absorbance of the control reaction and A sample is the absorbance of the test compound. The values of inhibition were calculated for the various concentrations of the sample. Ascorbic acid was used as positive control (Kamala et al., 2015) and all the tests were carried out in triplicate.

Nitric oxide assay and SEM,EDX :

The Griess Ilosvay reaction was used to evaluate nitric oxide radical inhibition. In this study, 1-naphthylamine (5%), instead of naphthyl ethylenediamine dihydrochloride (0.1% w/v), was used to typically modify the Griess Ilosvay reagent. The reaction mixture (3 ml) was incubated at 25°C for 150 minutes. It contained 2 ml of 10 mM sodium nitroprusside, 0.5 ml of saline phosphate buffer, and 0.5 ml of standard solution or aqueous and ethanolic extracts (500–1000 g/ml). After the reaction mixture had been incubated, 0.5 ml of it was combined with 1 ml of the sulfanilic acid reagent (0.33% in 20% glacial acetic acid), and the combination was let to stand for 5 minutes to allow the diazotization process to finish.

The percentage inhibition was calculated using the formula:

$$\% \text{ scavenging activity} = [(\text{Acontrol} - \text{Atest Or Astd})/\text{Acontrol}] * 100$$

where Acontrol is the absorbance of control and Atest Or Astd is the absorbance of test or standard, respectively.

Results:

The absorbance graph showed that the amount of light absorbed was at peak with wavelength of 579 nm.Since absorption is directly proportional to the concentration of the substance , the concentration of extract was highest at this point(figure 1).SEM imaging showed that the particle size of copper nanoparticles was between 40 nm–60 nm in size and X-ray diffraction analysis confirmed the amorphous nature of the synthesised copper nanoparticles(Figure 2). On assessing the DPPH assay and nitric oxide assay,it can be observed that the ability to scavenge the DPPH radical i.e percentage inhibition increased as the concentration of extract increased , same was the case in nitric oxide assay also, the standard chosen for the both the assays was Ascorbic acid, even though ascorbic acid showed more obvious inhibition percentage and thus antioxidant activities, Padina Gymnospora mediated copper nanoparticles were also no less potential , added to the fact that it is a more biotic component, with enhanced antioxidant properties due to the presence of copper nanoparticles.(Figure 3 and 4).

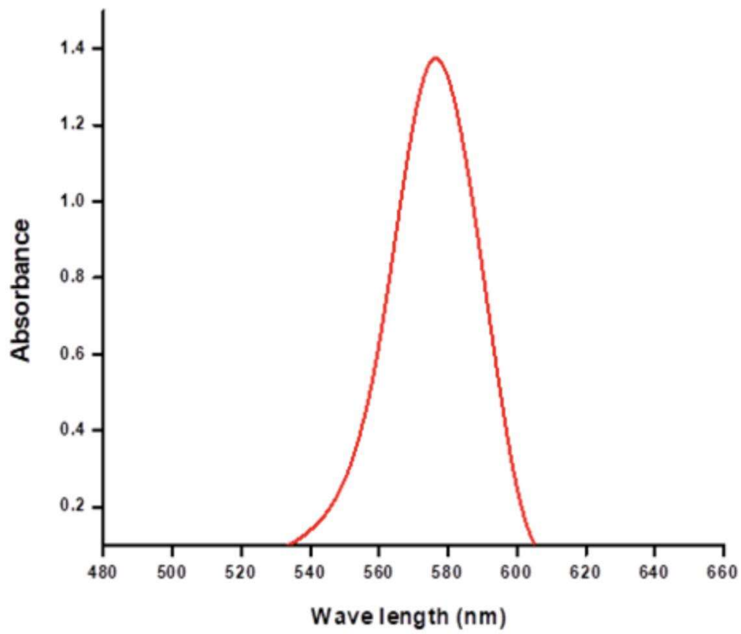

Fig 1. Absorbance of extract in nanometers

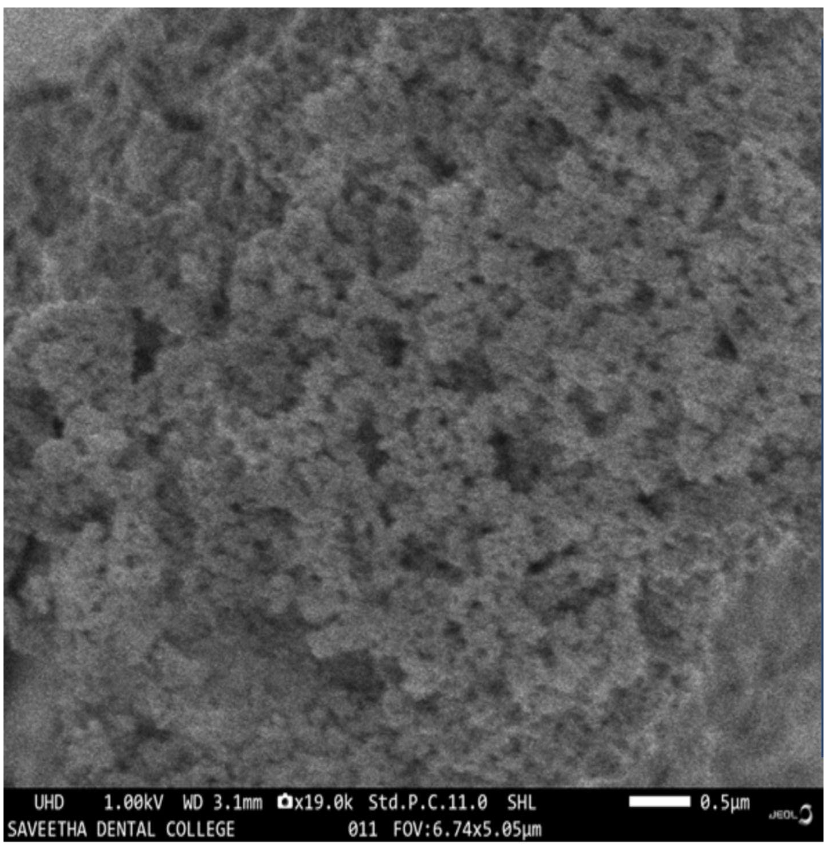

Fig 2. SEM imaging of synthesised Padina Gymnospora mediated copper nanoparticles

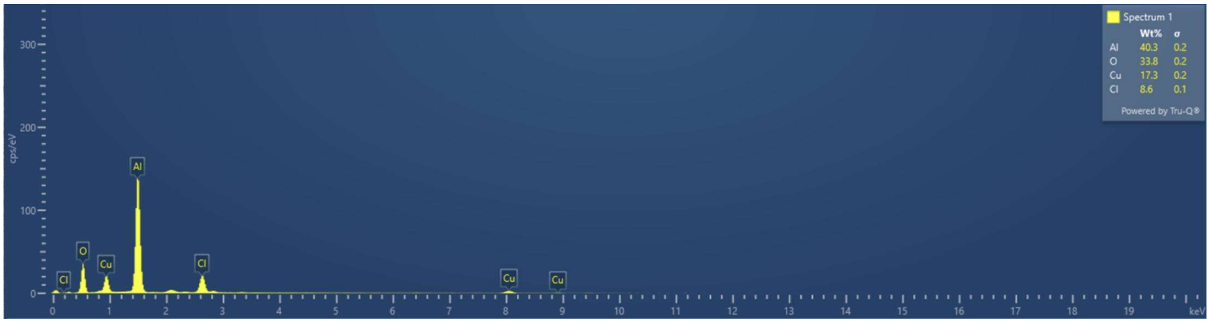

Fig 3. EDX imaging

DPPH assay		STD Ascorbic acid	NITRIC OXIDE ASSAY		STD Ascorbic acid
concentration(µg/ml)	samples	Std	concentration (µg/ml)	samples	Std
25	16.5±1.8	24.5±1.5	25	17.5±2.6	23.6±2.3
50	22.7±2.2	35.6±2	50	28.3±2.4	42.1±2.2
75	36.2±2.4	52.5±1.8	75	36.7±2.8	52.8±1.8
100	50.8±2.7	70.6±2.2	100	52.6±2.6	72.5±2.4

Fig 4.1. Table showing DPPH assay and Nitric oxide assay values in relation with standard (Ascorbic acid)

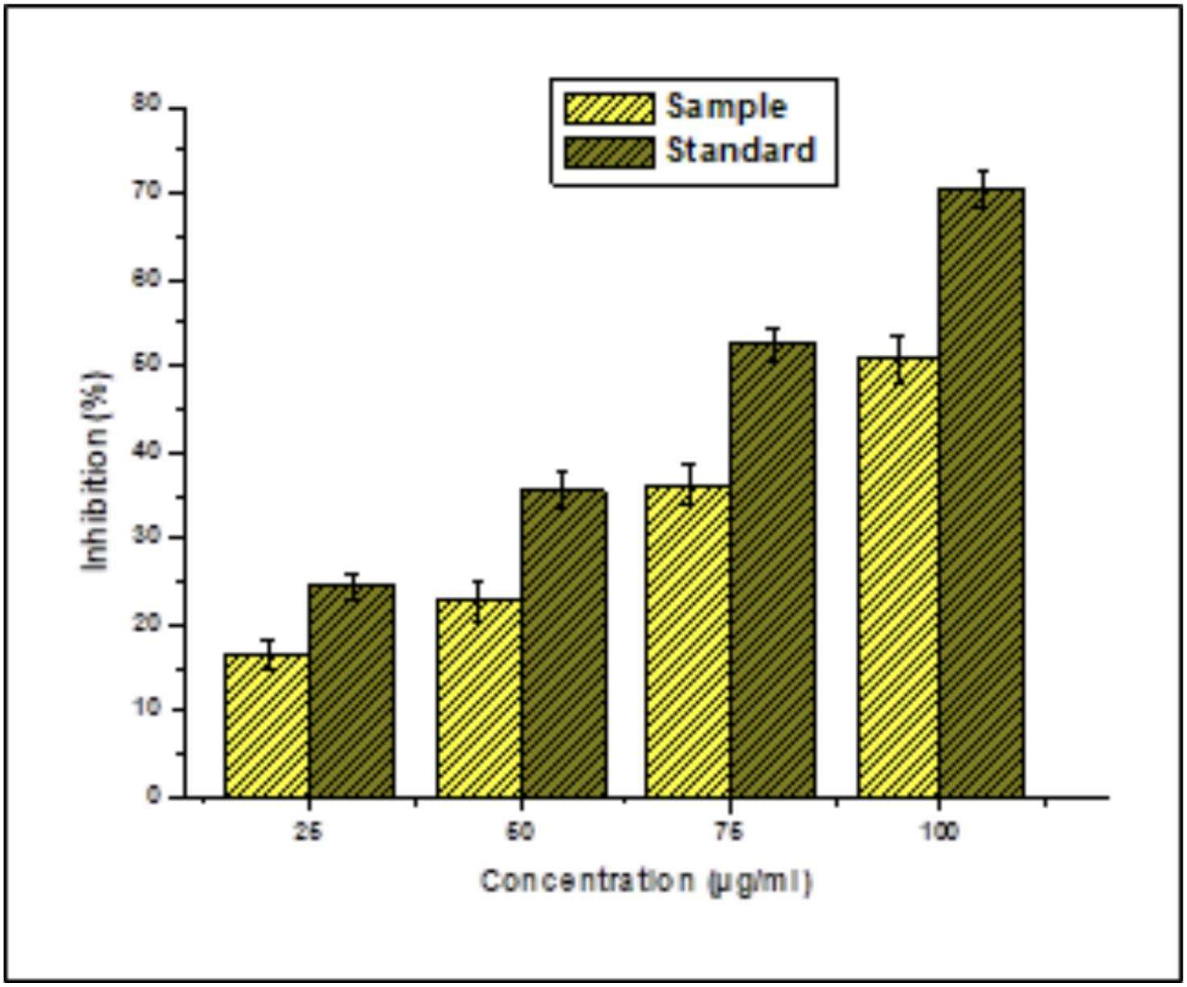

Fig 4.2. Bar graph represents the antioxidant effect of the P.gymnospora seaweed ethanolic extract. The X-axis represents various concentration levels and the Y-axis represents the percentage of the Nitric oxide inhibition, data implies as mean‡SEM

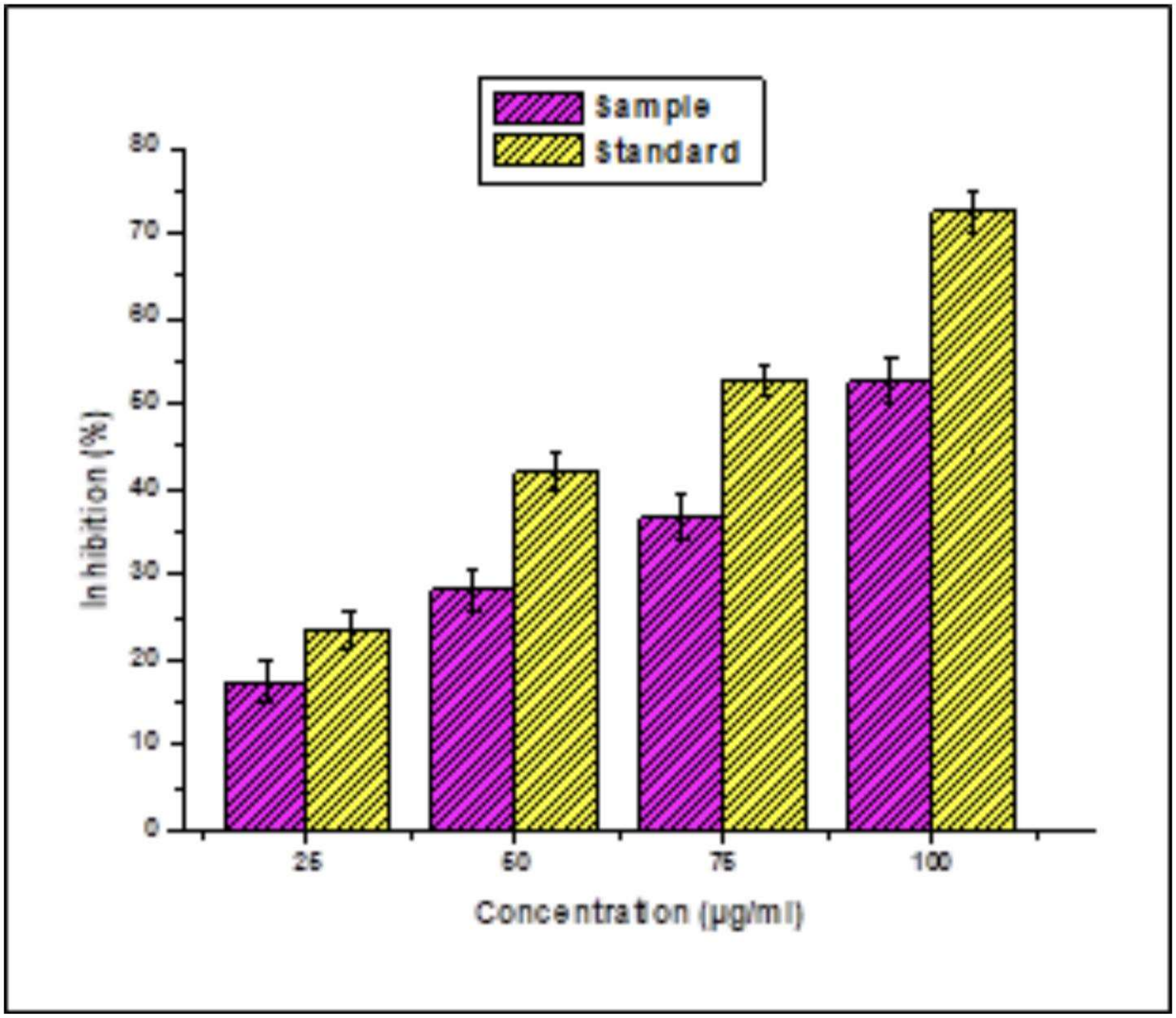

Fig 4.3 Bar graph represents the antioxidant effect of the P.gymnospora seaweed ethanolic extract. The X-axis represents various concentration levels and the Y-axis represents the percentage of the DPPH inhibition, data implies as mean‡SEM

Discussion :

Free radicals are created by several metabolic pathways in humans. Due to the release of reactive oxygen species that interact with molecules in other unrelated metabolic pathways, the free radicals are unstable and result in cellular damage. Free radicals are therefore to blame for a variety of degenerative diseases and lowered immune function. By removing electrons from the abundant reactive oxygen species and scavenging oxygen-derived free radicals, antioxidants protect against diseases(10)

In a different investigation by Bipin et al. 2021, the DPPH test was carried out using a methanolic extract of the seaweed Padina gymnospora. When compared to the standard, the antioxidant potential of the herbal extract was significantly different.

In yet another study conducted by Kenneth et al 2022 the biosynthesized CuONPs' antioxidant activity against the DPPH radical was assessed, and its efficacy was contrasted with that of CSE, PAE, and conventional ascorbic acid. In line with the plant extracts and the standard, the percentage scavenging activity of green produced CuONPs rose with increasing CuONPs concentration. As a result, the greatest concentration of the antioxidants applied reported the highest antioxidant activity.

Which were similar to the results observed by the study done by Das et al 2020 ,the percentage of antioxidant activity of the M. oleifera leaves extract and the produced copper nanoparticles was evaluated using the DPPH test against ascorbic acid as a reference. The generated copper nanoparticles demonstrated antioxidant activity.

Though no study is done yet on Padina Gymnospora mediated copper nanoparticles, the results of the above mentioned similar studies , were in correlation to the results of the present study.

Conclusion :

A sustainable and environmentally responsible method of utilising the potential advantages of nanotechnology in the realm of antioxidants is to use marine algae, specifically Padina Gymnospora, as a bioresource for the production of copper nanoparticles.The copper nanoparticles derived from Padina Gymnospora demonstrated potent antioxidant activity.

Overall, the results of this study show that copper nanoparticles mediated by Padina Gymnospora marine algae have significant potential as a possible therapeutic agent for treating oxidative stress-related illnesses and age-related problems. To determine their safety, effectiveness, and prospective uses in the biomedical and pharmaceutical areas, more in-depth research, including in vivo investigations and biocompatibility evaluations, are required. These nanoparticles may grow into useful weapons in the struggle against oxidative stress-related health problems with further study and development.

Conflict of interest:
The author reported the conflict of interest while performing this study to be nil.

Ethical clearance:

This study does not require an ethical approval number as it is in an in Vitro study.

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