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Potentiating effect of ecofriendly synthesis of copper oxide nanoparticles using brown alga: antimicrobial and anticancer activities

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Abstract. **This study reports the *in vitro* antimicrobial and anticancer activities of biologically synthesized copper nanoparticles. The antimicrobial activity of green synthesized copper oxide nanoparticles was assessed by well diffusion method. The anticancer activity of brown algae-mediated copper oxide nanoparticles was determined by MTT assay against the cell line (MCF-7). Maximum activity was observed with *Pseudomonas aeruginosa* and *Aspergillus niger*. Effective growth inhibition of cells was observed to be more than 93% in antibacterial activity. Thus, the results of the present study indicates that biologically synthesized copper nanoparticles can be used for several diseases, however, it necessitates clinical studies to ascertain their potential as antimicrobial and anticancer agents.**

Keywords. **Antibacterial; anticancer activity; copper oxide nanoparticles; *Sargassum polycystum*.**

1. Introduction

Nanotechnology and nanoparticles-based products and their applications are increased now-a-days due to their biological effectiveness. It is well known that inorganic nanomaterials are good antimicrobial agents. Current research on bactericidal nanomaterials has opened a new era in pharmaceutical industries and environmental society [1]. Among the various nanoparticles, metal nanoparticles assume special importance because they are easier and cheaper to synthesize and are the most promising in applications [2].

Unique properties such as electronic, magnetic, catalytic and optical properties are exhibited by metal nanoparticles which are different from those of bulk metals. These properties would result in interesting new applications of metal nanoparticles that could potentially be utilized in biomedical sciences and areas such as optics and electronics [3]. Thus, to make use of such nanoparticles, biosynthesis of nanoparticles is now established as an alternative to chemical and physical methods of synthesis [4].

Algae are otherwise called ‘bionanofactories’ because they synthesize nanoparticles with high stability, which are easy to handle and eliminate cell maintenance [5]. Algae are the naturally available plants, which are important sources of phytochemicals involved in the production of metallic nanoparticles. Recently, gold nanoparticles synthesized using the extract of algae such as *Sargassum wightii* [6], *Turbinaria conoides* [7], *Laminaria japonica* [8] and *Stoechospermum marginatum* [9] have been reported.

Among the marine sources, the macroalgae (seaweeds) occupy a significant place as a source of biomedical compounds. The compounds derived from macroalgae are reported to have a broad range of biological activities such as antibacterial [10], anticoagulant [11] and antifouling activity [12]. Seaweeds have been used since ancient times and have an exclusive place in traditional medicine of maritime nation as vermifuges, aesthetics and antibiotics in the treatment of cough, wounds, gout, goiter, hypertension, venereal diseases, cancer and a variety of other sicknesses [13].

This study mainly aims in assessing the anticancer and antimicrobial activities of green synthesized copper nanoparticles from brown seaweed *Sargassum polycystum*.

2. Materials and methods

2.1 *Study area*

The study area chosen for the collection of seaweeds is Gulf of Mannar (Rameshwaram) biosphere reserve which is situated in the southern part of the peninsular India, this extends from Adams bridge to Cape Comorin which is remarkable for diversity of flora and fauna and it also sustains a good fishery.

2.2 *Collection of seaweed and microorganisms*

Samples were collected from shallow waters of the Gulf of Mannar region (Lat. 9°17′N Long. 79°17′E) at Mandapam, southeast coast of India. The collected seaweeds were washed with salt water to remove all the epiphytes and then allowed in distilled water for about 15 min to remove the salts. Initially, the seaweed samples were authenticated by Botanical Survey of India (BSI), Coimbatore (BSI/SRC/5/23/2014-15/Tech.169), India. Thus, the collected brown

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seaweed was identified as *Sargassum polycystum* C. Agardh. Subsequently, the seaweeds were air dried, then cut into small pieces and made into powder and used for further studies.

Bacterial pathogens namely *Shigella dysenteriae* MTCC 5151, *Escherichia coli* MTCC 1302, *Pseudomonas aeruginosa* MTCC 2297, *Salmonella typhimurium* MTCC 1254 and *Vibirio cholerae* MTCC 3906, and fungi pathogens *Aspergillus oryzae* MTCC 5431, *Aspergillus niger* MTCC 2232, *Candida albicans* MTCC 184, *Aspergillus flavus* MTCC 2260 and *Aspergillus nidulans* MTCC 434 were collected from the Department of Microbiology, Karpagam University, Coimbatore, Tamil Nadu, India.

2.3 *Synthesis of copper nanoparticles*

To synthesize copper nanoparticles, *Sargassum polycystum* aqueous extract was added to 100 ml of 1 mM aqueous copper solution in a 250 ml Erlenmeyer flask. The flask was then kept overnight at room temperature. The Cu nanoparticle solution thus obtained was purified by repeated centrifugation at 12,000 rpm for 15 min followed by re-dispersion of the pellet in deionized water. Then, the Cu nanoparticles were dried in oven at 80°C [14].

2.4 *Antimicrobial activity*

Antimicrobial activity of the copper oxide nanoparticles was determined by the agar well diffusion method. Pathogens were seeded on Muller Hinton agar for bacteria and potato dextrose agar for fungi by using sterilized cotton swabs. Agar surface was bored by sterilized gel borer to make wells (5 mm diameter). Tetracycline was used as positive control for bacteria and amphotricin for fungi. Different concentrations of the sample were maintained (10, 20, 30, 40 and 50 μg) for both bacteria and fungi. Plates were incubated for 24 h at 37°C (bacteria) and for 48 h at room temperature (fungi). Triplicate plates were maintained for each organism. The diameter of such zones was measured and the mean value for each organism was recorded and expressed in millimetre [15].

2.5 *Anticancer activity by MTT assay (3(4,5-dimethylthazol-2-yl)-2-5-diphenyl tetrazolium-bromide)*

A human breast MCF-7 cell line (cell culture) was obtained from the National Centre for Cell Science (NCCS), Pune, India. The cells were continued in Eagles minimum essential medium (EMEM) added with FBS (10%, v/v) at 37°C in a CO_2 incubator (95% air, 5% CO_2 and 100% relative humidity). To evaluate cytotoxic effect of the biologically synthesized copper nanoparticles against MCF-7 cells, which were collected in the exponential stage of growth, seeded into 96-well plates (15,000 per well) and permissible to adhere for 48 h. Then, different concentrations (6.5, 12.5, 25, 50, 100 μg ml^{-1}) of *Sargassum polycystum*-mediated copper nanoparticles were added to the desired wells and incubated for 48 h. A 20 μl of EMEM medium having MTT (5 mg ml^{-1}) was added to each well and incubated for 4 h at 37°C. Later, the medium was altered with 100 μl of DMSO, and optical densities were measured at 570 nm. All the measurements were made in triplicate and expressed as the mean ± standard error.

3. Results and discussion

The study reveals a simple and high yielding method for the synthesis of copper nanoparticles from the brown seaweed *Sargassum polycystum* using copper sulphate as the precursor. Synthesized copper nanoparticles exhibited antibacterial activity (±17 mm) [16]. Mostly seaweeds are an excellent source of biocomponents such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols and carotenoids which exhibited different biological activities which are indirectly responsible for the synthesis of copper nanoparticles. Nanoparticle synthesis mainly depends on the reaction time and phytochemical components of the algae extract [17].

In this study, antibacterial activity of the Cu nanoparticles exhibited maximum activity in *Pseudomonas aeruginosa* (15 ± 0.5 mm), while the lowest activity was observed in *Shigella dysenteriae* (6±0.5 mm) (figure 1) among the different bacterial pathogens used. According to Faulkner [18] maximum activity was exhibited in *P. aeruginosa* with 13 mm inhibition zone and *V. cholerae* with 11 mm zone of inhibition. Also Blunt *et al* [19] reports indicate that *Nitraria schoberi*-mediated synthesis of copper nanoparticles have maximum inhibition activity observed with *P. aeruginosa*, *V. cholerae* and *E. coli*.

Antifungal activity of the present study reveals that maximum inhibitory activity was observed with *Aspergillus niger* (20 ± 0.5 mm) (figure 2). According to the previous reports, Karmegam *et al* [20], *Magnolia grandiflora*-mediated synthesis of copper nanoparticles had maximum inhibition activity with *Aspergillus niger* which had 3% higher activity (26 mm) than *Zingiber officinale*-mediated copper nanoparticles when compared with the reports of Sangeetha and Saravanan [21] and Harne *et al* [22]. Minimal inhibition activity in this study was observed with *Aspergillus oryzae* (12 ± 0.5 mm). These results were supported with some previous reports of Bhacuni and Rawat [23] which suggests that copper nanoparticles synthesized from *Tritium aestivum* exhibited similar inhibition activity with the maximum zone of 18 mm for *Aspergillus niger*. Likewise, minimum inhibition activity by *Aspergillus flavus* was 9 mm zone [23].

The cytotoxicity of the copper nanoparticles was evaluated against MCF-7 breast cancer cell lines at various concentrations (6.5–100 μg ml^{-1}). Figure 3 shows the cytotoxic activity of copper nanoparticles. The IC_{50} value for

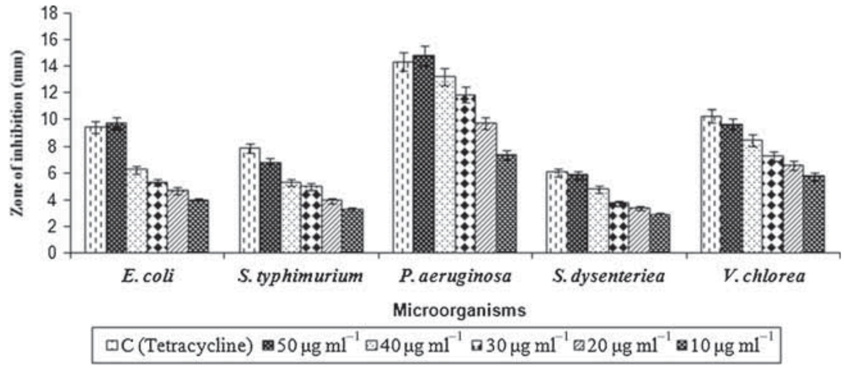

Figure 1. Antibacterial activity of copper nanoparticles. Values are expressed as mean ± SD of the three replicates.

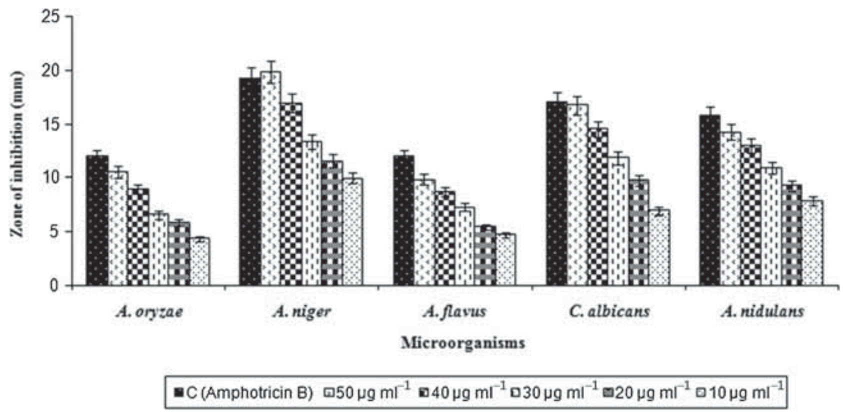

Figure 2. Antifungal activity of copper nanoparticles. Values are expressed as mean ± SD of the three replicates.

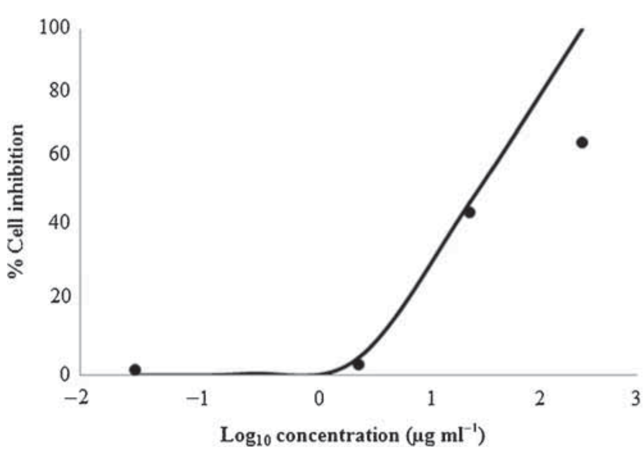

Figure 3. Anticancer effect of *Sargassum polycystum*-mediated copper nanoparticles on MCF-7 cell lines.

copper nanoparticles was 61.25 μg ml^{-1}. Maximum concentration of copper nanoparticles (100 μg ml^{-1}) effectively inhibited the growth of cell by more than 93%. Harne *et al* [22] reported the anticancer property of copper nanoparticles against MCF-7 human breast cancer cells.

4. Conclusion

Sargassum polycystum shows maximum antimicrobial and anticancer activities against different pathogens used. Further studies should be made to identify and evaluate the actual substances responsible for the property. The results from the present study revealed that significant antimicrobial and anticancer activities were exhibited by *Sargassum polycystum*-mediated copper nanoparticles.

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