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Original article

Biogenic silver nanoparticle synthesis with cyanobacterium *Chroococcus minutus* isolated from Baliharachandi sea-mouth, Odisha, and *in vitro* antibacterial activity

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ABSTRACT

The burden from microbial diseases has to be addressed in an increasing pace due to multidrug resistance, causing clinical annoyance, globally. Indeed, employing eco-friendly approaches by green synthesis of nanoparticles with metals/metalloids as antibacterial compounds would be continual and cost-effective. Herein, the synthesis of silver nanoparticles (AgNPs) by aqueous extracts of the cyanobacterium *Chroococcus minutus* (strain, CRLSUM10), collected from the meeting point of sea and river, East coast of India, are presented; both reducing and stabilizing mediators of the synthesized AgNPs were ascertained, by characterization by UV–visible spectrophotometry, XRD, FTIR, SEM and SEM-EDX. Furthermore, synthesized AgNPs had a remarkable antibacterial activity at the lowest dose, 100 mg against pathogenic strains of *E. coli* and *S. pyogenes*, rendering those as novel antibacterial agents against 'upper respiratory tract infection'.

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1. Introduction

The upper respiratory tract (URT) infection (URTI), known as "common cold", grossly includes infections by rhinovirus, bacterial flora with *Escherichia coli* and *Streptococcus pyogenes* striking nose, sinuses, pharynx and larynx. Virus infections are general, while other less frequently infecting agents are bacteria, fungi or helminthes (Barrie and Gallacher, 1975; Masavkar and Naikwadi, 2016). URTI is frequent with playgroup children (6–12 yr age), while it is less frequently experienced, viz., 2–4 URTI episodes per year with adults. In India, the annual attack rate between 15% and 42% in pre-school and school-aged children. Infections are mainly air-borne; but by direct contact, it spreads more often than assumed. Specifically, 17.2 billion cases of URTIs are estimated to have occurred in 2015 alone, as in 2014, those caused

about 3000 deaths approximately, down from 4000 decimations in 1990 (Vos et al., 2016).

Control of infection today has become an uphill task due to bacterial drug resistance. Because of Darwinian/ natural evolution in bacterial consortia, there occurs a continual upgradation of the genome with each pathogenic bacterium. Consequently, newly introduced antibiotics fail progressively. Moreover, there is natural genetic exchanges operative in bacterial consortia; this leads to a situation, an antibiotic never applied against a particular bacterium in 'antimicrobial stewardship program', is often found resistant to it. Thus, this type of situation everywhere would create grand failures in clinical control of bacterial diseases. Each physician pragmatically takes a preemptive attempt of administration of some higher level of antibiotic, which is essential for clinical managements in control of an ongoing infection or that after a surgical episode. Indeed, the phenomenon of development of antibiotic resistance in any bacterium further repeats itself for each newly introduced antibiotic. Unfortunately, the use of any higher level of antibiotics remains repugnant as it triggers several grimy non-target health hazards raising far-reaching adverse conditions in public health, by spreads of multidrug resistant bacteria. In this scenario, the development of an effective drugable compound is an obsessive quest of the day; for example, as seen for leprosy (Swain et al., 2018).

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Ethnically, natural products are being used to overcome infections by extracts of ginger, turmeric, honey, garlic, echinacea, lemon juice, other fruit juices, and modern probiotics. Moreover, cyanobacteria (blue-green algae) have several bioactive compounds such as, pigments, phycoerythrin, phycocyanin, allophycocyanin, carotenoids, etc., which lend themselves as antiviral, antibacterial, antifungal and anticancer compounds basically (Singh et al., 2005), and a systematic screening of cyanobacterial compounds or cyano-compounds would give a way to locate new drugable entities (Sahoo et al., 2019b). Several cyano-compounds from Chroococaceae, Nostocaceae, Oscillatoriaceae have remarkable antibacterial activities (Soltani et al., 2005; Anwer and Abdulkareem, 2014; Ghasemi et al., 2007).

Indeed, cyanobacteria (photosynthetic prokaryotes) are identified as rich sources of biologically active anti-cancer compound viz., norharmane isolated from *Chroococcus minutus* (Sahoo et al., 2019a), in addition of pigments. These occur at a diversified in a range of habitats viz., freshwater, terrestrial, rocky shores, hot springs and euphotic zone of oceans. Those have tremendous capabilities to overcome environmental stresses, UV-exposure, desiccation and flooded waters, temperature and high salinity (McGregor et al., 2007; Karan et al., 2017). Furthermore, cyano-compounds lend themselves in human nutrition (Thiel et al., 1989) and therapeutic purposes (Vijayakumar and Menakha, 2015; Sahoo et al., 2019a, 2019b). Thus, the cultivation processes of particular cyanobacterial strains for mass production is actively considered among the few belonging to orders, Chroococcales, Nostocales, and Oscillatoriales, for example. It was known that *Chroococcus minutus* (Chroococcales) can bio-accumulate nonylphenol from the ambient medium (Gademann, 2007).

There are two basic methods in synthesis of metallic nanoparticles, 1. Chemical method with expense of conventional energy sources involved from physico-chemical reactions. This process required chemical process which generates mild/strong toxic byproducts; consequently, this process is not environmentally friendly (Jenck et al., 2004), 2. Green synthesis method with utilization of metabolic/photosynthetic energy generated in living cell of synthesis of nanoparticles with an organism. This process is cost effective and environmental friendly (Ahmad et al., 2019; Abdel-Raouf et al., 2019). In this perspective, there is a demand of antimicrobials without any unwanted side effects on host. Silver based nanoparticles (AgNPs) are often reported having *in vitro* control of pathogenic bacteria and used as therapeutic purposes (Dos et al., 2014). It was intuitive to attempt for green synthesis of AgNPs with a bacterium with photosynthetic ability could serve the purpose of newer antibacterial and lesser host toxicity. Biogenic synthesis of AgNPs has become a popular pursuit as silver is basically considered as an antimicrobial metal but, green synthesis of AgNPs with *C. minutus* has not been described.

There is an inherent problem of identification of a particular alga from brackish water, which is a dynamic mixture of fresh and marine waters supporting a unique algal flora. This was solved presently both by traditional microscopic and molecular identification methods. In the present study, physico-chemical characteristics of biosynthesized AgNPs with *C. minutus* namely, UV-visible spectrophotometry, (XRD) X-ray diffraction for powder structure, (FTIR) Fourier transform infrared for interaction of nanoparticles at various ranges, scanning electron microscope (SEM) and SEM-energy Dispersive-X-ray (SEM-EDX) for surface structure of AgNPs were determined. From these studies, it could be confirmed that green synthesis of these AgNPs was time dependent. Furthermore, the antibacterial activities of AgNPs could help in assessing the control of pathogenic bacteria namely, *E. coli*, *S. aureus* and *P. aeruginosa*. Thus, this study demonstrated that green synthesis/biosynthetic approach of AgNPs could be newer antibacterial agents for the control of URTI, in future.

2. Materials and methods

2.1. Isolation, ultra-structure analysis and culture conditions

The brackish water planktonic samples were collected in November–December 2017 in 'new confluence of sea and a rivulet' in Puri district, East coast of Odisha state (Lat. 19.7568 N; Log. 85.6962 E) (Fig. S1). Aliquots of BG-11 medium were poured into Petri plates as the selective step from a mixed natural flora; for a unialgal-axenic culture of *C. minutus*, the streak plate method was carried out serially for seven generations.

Furthermore, samples were observed with a Magnus research microscope connected with the Olympus E 520 digital camera. Algal cells were measured at several magnifications, $\times 20$, $\times 40$ and $\times 100$, with further morphological confirmation using a standard Indian flora, and 'Algal morphology webs' 2018 (Anonymous, 2018). Samples were monitored with the most morphological characteristics and the individual taxa were observed under a scanning electron microscope (SEM) and molecular identification by 16SrRNA sequence analysis.

Inoculants of *C. minutus* were directly poured into 100, 500, 2000, 5000 ml volumes of BG-11 medium for scaling up biomass, which were harvested after each growth cycle of 10–16 days. Growth of *C. minutes* was detected with a double beam spectrophotometer (Systronics 2203), at different phases of growth. The growth conditions were previously detailed (Thiel et al., 1989; Komarkova et al., 2010; Mohan et al., 2010; Karan et al., 2017).

2.2. Biosynthesis and spectral analysis of AgNPs of *C. minutus*

Harvested log phase cell-biomass of *C. minutus* was mixed as dried powder (20 mg) with an aliquot of 20 ml sterile water at 40 °C for 24 h. Thereafter, the AgNO₃, 2 mg/2ml at concentration (1 Mm) was added at 30 °C to the aqueous extract; the blank AgNO₃ solution was the reference control. Another aliquot of fresh AgNO₃ solution was further added to filtered of the whole mixture in 2000 ± 200 lx fluorescent light was kept for 24–56 hr. The incubated mixture appeared pale yellow to deep brown with AgNPs; NP synthesis was confirmed by spectral reading at 200–750 nm OD by UV-Vis spectrophotometry. Thereafter, centrifugation of the mixture with AgNPs at 9000 rpm for 10 min was done; the supernatant discarded and to the pellet was suspended in 10 ml sterile water for 5 times repeatedly. The lot was dried at 30 °C before spectral characterization (Husain et al., 2015; Abdel-Raouf et al., 2019).

2.2.1. UV-VIS spectroscopy

The reduction of silver ions was monitored by sample of 2 ml aliquots by measuring at several intervals, in the range of intensity at 300–750 nm.

2.2.2. SEM-EDX analysis

Scanning electron microscopy- energy-dispersive X-ray (SEM-EDX) analysis using Hitachi, S3400N model were studied in small amounts of biomass carbon coated with copper grid for surface morphology.

2.2.3. XRD analysis

The structural phase identification of diffraction patterns (Rigaku, UltimaN) was executed by powder diffractometer 2 theta range from 5–80° at 2° per minute.

2.2.4. FTIR spectroscopy

Attenuated total reflectance- Fourier transform infrared (ATR-FTIR) analysis (JASCO FT/IR4600-ATR) was done for evaluation for

functional groups in chemical composition of AgNPs, at spectral range 400–4000 cm^{-1} .

2.3. Antibacterial study of *C. minutus*-AgNPs

Antibacterial activity of AgNPs was assessed using agar-well diffusion method, with sterile nutrient agar medium and pH at 7.4. In each agar plate four punched wells were filled up with graded levels of synthesized AgNPs and a standard antibiotic gentamycin (Sahoo et al., 2019), which were incubated at 37 °C for 48–72 hr for measurement of inhibition zones (Baral et al., 2019).

2.4. Minimum inhibitory concentration (MIC)

Synthesized AgNPs of *C. minutus* were monitored at several graded concentrations, 1, 5, 10, 20, 50, 100, 200 and 500 mg/ml in DMSO solution, individually. Moreover, fresh nutrient broth was prepared and added serially to each 80 μl aliquot of cited dilutions of AgNPs, along with aliquot of 20 μl pathogenic strains of bacteria, *S. aureus*, *Pseudomonas aeruginosa* and *E. coli* along with 80 μl aliquots of nutrient medium onto a sterile 96- MIC well plate. In the first column of the plate, DMSO solution served as the negative control, followed by test samples. After incubation at 37 °C

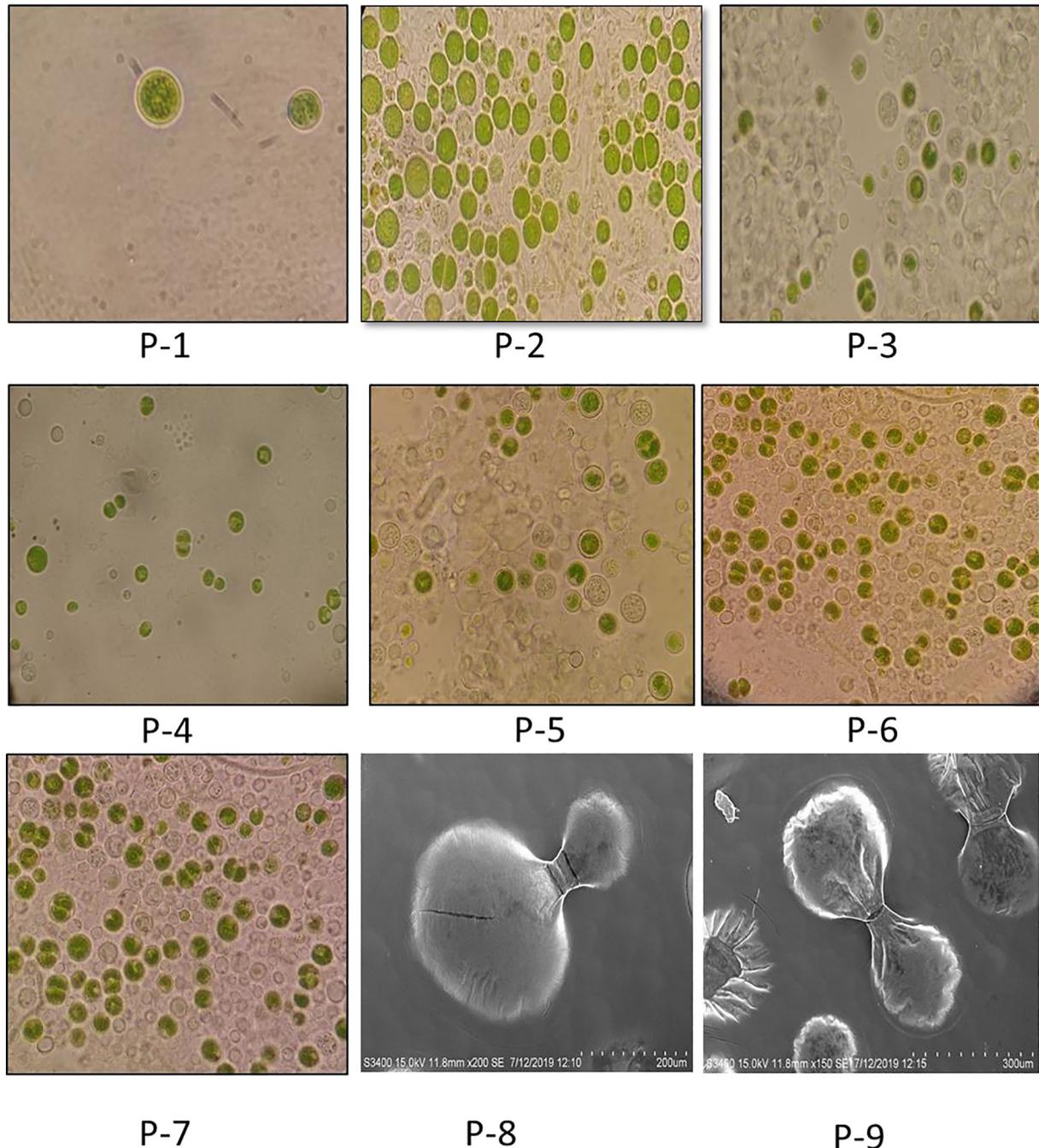


Fig. 1. (Plate 1–9): 1. *C. pallidus*. 2. *C. indicus*. 3. *C. cohaerens*. 4. *C. minutus*. 5. *C. montanus*. 6. *C. macrococcus*. 7. *C. schizodermaticus*. 8–9. Scanning electron microscope of *C. minutus*.

for 72 hr, an aliquot of 5 μ l TTC was added to each well for color changes due to bacterial growth inhibition (Nayak et al., 2015).

3. Results and discussion

3.1. Cyanobacterial diversity of Baliharchandi sea-mouth

Presently, morphological determination of seven isolates of *Chroococcus* sp. collected from brackish water, Puri, Odisha were done (Fig. 1.; Fig. S3). Those belong to Chroococcaceae family; the taxonomical details *Chroococcus* sp. are given:

3.3.1. Genus: *Chroococcus*. Cells are spherical, subspherical and / or hemispherical, distinct sheath of individual cell, colonies free swimming, colony merged/ attached, lamellated, homogeneous, irregular size lead to broken, reproduction by cell division and colony fragmentation.

1. *C. pallidus* Nag: Thallus gelatinous, pale yellow to dark yellow, cell singular and 2–4, oblong colonies, with sheath 5–8.5 μ , without sheath 7.5–12 μ broad, colorless sheath, tick, unstratified.
2. *C. indicus* Zeller: Thallus gelatinous, thin layer, light brownish, single cell, oblong to sub spherical, 3.2–7.6 μ diam., greenish sheath, hyaline layer, granular.
3. *C. minutus* (Kutz) Nag: Cell spherical or sub-spherical, light or dark green, single and 2–4 in a group, sheath 5–12 μ , without sheath 5–11 μ , rarely lamellated.
4. *C. cohaerens* (Breb) Nag: Thallus slimy and some gelatinous, dark green, single cell and 2–8 in group, sheath 5.5–12 μ , without sheath 4–11 μ , sheath thin, unlamellated.
5. *C. montanus* Hansgirg: Thallus slimy, gelatinous, brownish or dark green, single cell and 2–8 in group, sheath 4.5–12 μ , without sheath 5–13 μ , colonial sheath.
6. *C. macrococcus* (Kutz) Rabenh: Thallus mucilaginous, broad, yellowish to brown, cell spherical, without sheath 15–45 μ , sheath 20–50 μ colonial sheath.
7. *C. schizodermaticus* West: Thallus slimy, gelatinous, straw yellow to dark yellow, single cell and 2–8 in group, sheath 5–40 μ , without sheath 4–18 μ , outer layer often broken, lamellated.

Like any cyanobacterium, *Chroococcus* sp. is prokaryotic lacking of any of membranous organelles. Cells are spherical or subspherical with a diameter ranging between 0.4 and 40 μ m (Wood et al., 2017). These are morphologically similar to the green algae, but with a bluish cell masses; these are capable of living in an-oxygenic or saline waters, justifying occurrence in brackish waters. These occur mainly in a group of 2–4 or 8–16 celled colony-like structure. Cells are embedded in mucilaginous sheath each cell surrounded by their sheath also each cell has its cell wall (McGregor et al., 2007; Munir et al., 2016).

These are planktonic and grow in a wide variety of freshwater habitats, ponds, tanks, lakes and sometimes being attached to some submerged objects. Several forms grow in moist stones, bricks, wet soil and paddy fields (Kaushik, 2014); those also have been identified in water sources of higher salinity (Sompong et al., 2008). Moreover, *Chroococcus* sp. were grown in various media with environmental factors; structural morphology and 16S rRNA data are the key features to identify species, as an example *C. nageli* (Komarkova et al., 2010).

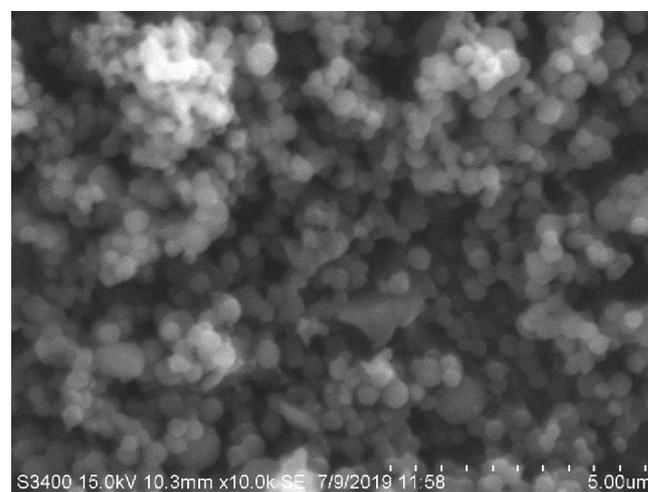


Fig. 3a. SEM micrograph of synthesized AgNPs.

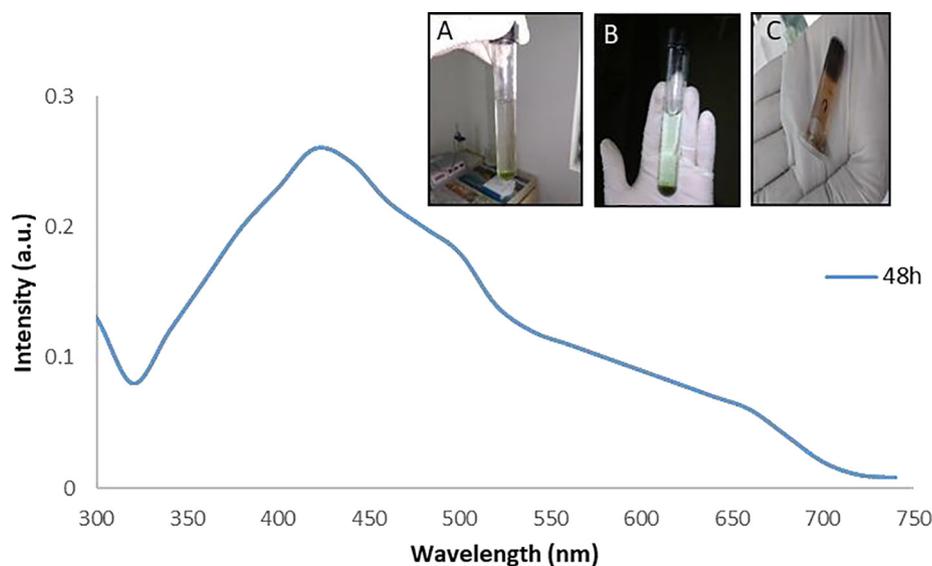


Fig. 2. UV/Vis spectra of silver nitrate with *C. minutus* recorded from the cultured supernatant after 48hr. Insert: (A) Culture tube containing *C. minutus* and AgNO_3 solution for 12hr (no colour change); (B) Culture tube containing *C. minutus* and AgNO_3 solution for 24hr (light brown colour); (C) Culture tube containing *C. minutus* and AgNO_3 solution for 48hr (deep brown colour).

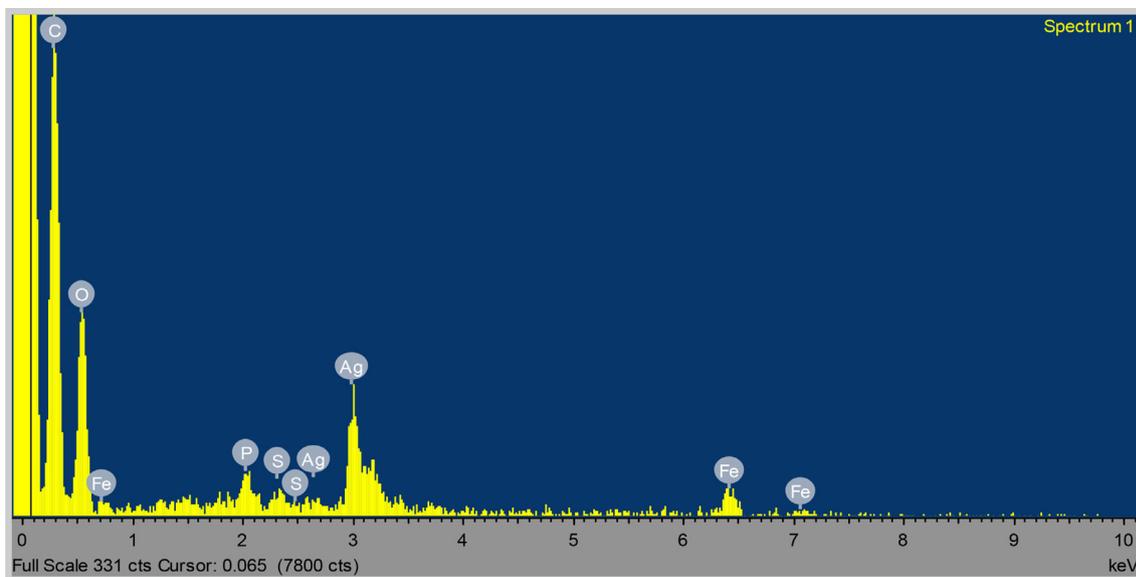


Fig. 3b. SEM-EDX micrograph of AgNP synthesis confirmed structure in the presence of Ag.

3.2. Biomass growth and synthesis of AgNPs

Furthermore, the OD values of broth cultures of *Chroococcus* sp. were observed in a steady-state of growth in alternate days for 14 days. The generated S-curve was usual during the growth period. Growth of *C. minutus* under 30 ± 5 °C was the log phase of growth; furthermore, in 3–4 growth cycles the cyanobacterial biomass was harvested (Fig. S3). Thereafter, the synthesis of AgNPs was carried out by using the cell extract and AgNO_3 .

3.3. Characterization of AgNPs

3.3.1. UV-Vis spectrophotometry

The formation of AgNPs was confirmed by visual observation of the mixture from pale yellow to deep brown color; the color change was due to the surface plasmon variation, and sharp peak was noted at spectral range of 420 ± 10 nm (Fig. 2). The rate of

reduction of Ag^+ ion converting to Ag^0 for the presence of cyanobacterial bioactive compound was acting as mediator of AgNP synthesizer at 48 to 56hr. Similarly, cyanobacterial extracts of *Spirulina* and *Arthrospira* AgNPs had yellowish brown color at 45 hr (Husain et al., 2015).

3.3.2. SEM analysis

Scanning electronic microscope (SEM) is an evident. SEM micrograph images were clearly depicted the synthesis of AgNPs. Furthermore, elemental surface samples of presence of Ag were confirmed by SEM-EDX along with carbon ferum, respectively (Figs. 3a, 3b).

3.3.3. XRD analysis

XRD analysis confirmed the synthesis of AgNPs and crystalline structure was formed at 5–80 theta ranges (Fig. 4). The obtained XRD patterns were observed at values, 10.5° , 38.37° and 64.4° in

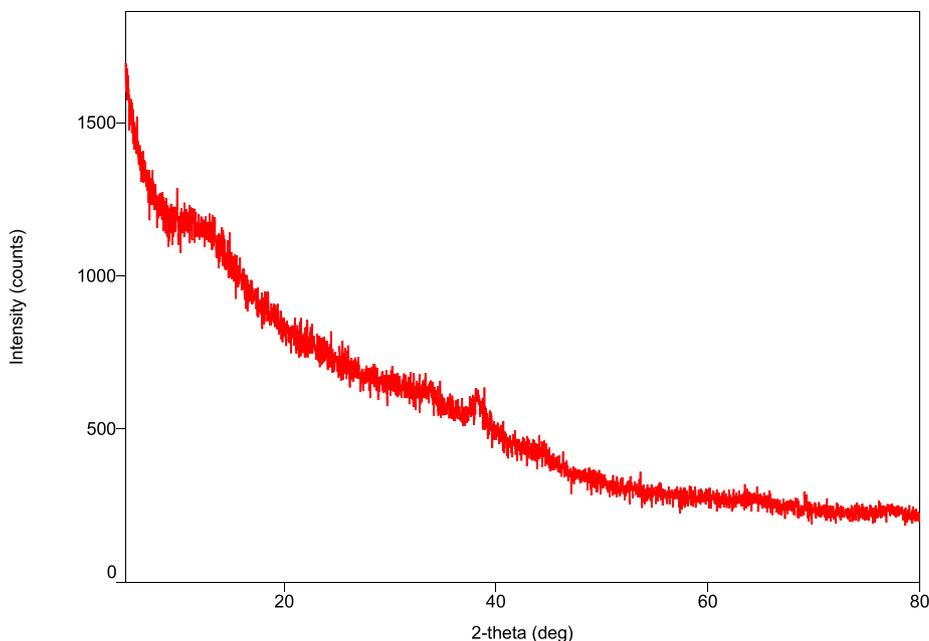


Fig. 4. XRD pattern of synthesized AgNPs with *C. minutus*.

correspondence to height values, 86, 67 and 20, respectively (Table S1). The formation AgNPs was confirmed by at 2-theta range, 38.37° and 64.4°; several studies had recorded patterns of XRD analysis at 38.2, 44.3, 64.6, 77.2° of the marine alga *Ecklonia cava*, for example (Venkatesan et al., 2016).

3.3.4. FTIR analysis

The FTIR analysis was confirmed by the presence of phenolic, methyl and alcoholic groups (Fig. 5) at following ranges, 3284.18 cm^{-1} (O-H str.), 2919.70 cm^{-1} (C-H), 1633.41 cm^{-1} (C = C str., N-H bend.), 1451.17 cm^{-1} (C-H bend.), 1375.96 cm^{-1} (O-H bend.), 1017.27 cm^{-1} (C-H bend.). Similarly, 1383.8 cm^{-1} was reported as silver reduction stages of alga, *Dictyota mertensii* (Fernandes-Negreiros et al., 2018).

3.4. Antibacterial activity

The synthesized AgNPs had zones of inhibition against, *E. coli* > *S. aureus* > *P. aeruginosa* at the range, 12–16 mm; and

MIC values were observed against *E. coli*, *S. aureus* and *P. aeruginosa* at 100, 200 and 100 mg doses of MIC, respectively (Fig. 6). Similarly, *C. turgidus* had an inhibitory effect against 3 bacterial pathogens, *E. coli*, *Salmonella typhimurium*, *Streptococcus faecalis*. Not only metabolites, exmetabolites of some species were reported as rich in antibacterial and antifungal (30–40 $\mu\text{g ml}^{-1}$) agent with similar richness as antifouling capability (Volk and Furkert, 2006; Hafez and Kibei, 2013). In the present situation, emulating antibacterial drugable agents against antibiotic resistant pathogenic bacteria is a dire necessity from natural sources (Lekshmi et al., 2016).

The biosynthesis of AgNPs with a microalga, the present cyanobacterium is coveted for the control of resistant bacterial pathogens. Ag is well known as having anti-bacterial properties; by the by, AgNPs synthesized from a non-conventional source could enhance the controlling capacity over pathogenic dreadful bacteria. This aspect might be worth pursuing further for the antimicrobial stewardship program. *Per se*, pathogenic microbes, as used here, are notoriously devastating because of their constant upgrading of the genus, resulting in consequent multidrug-

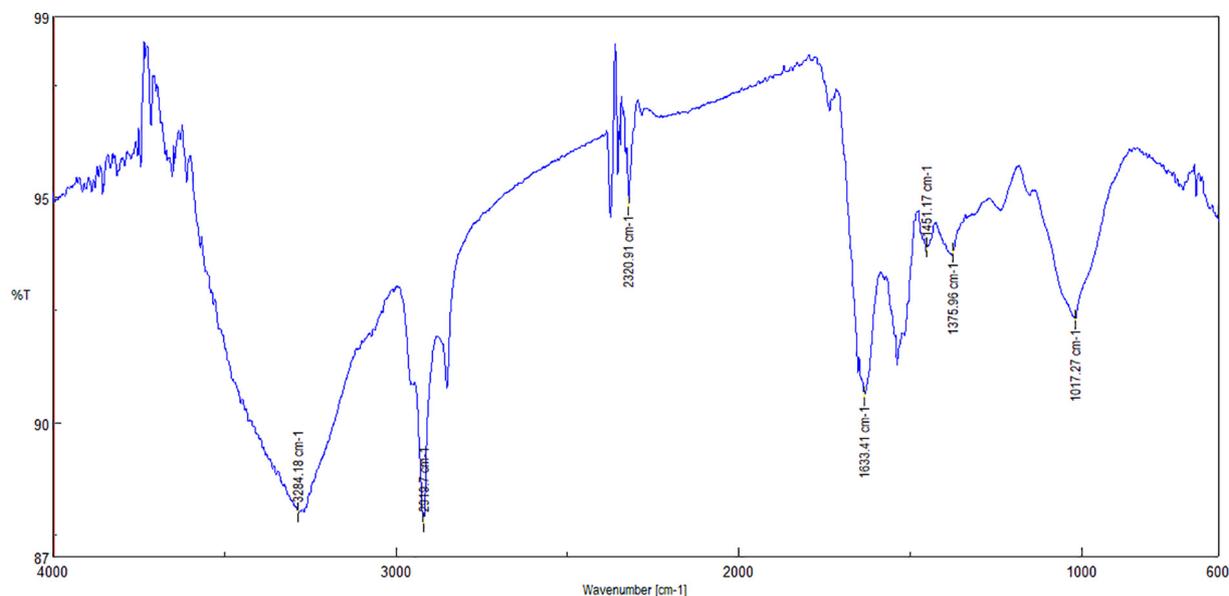


Fig. 5. FTIR spectrum of synthesized AgNPs.



Fig. 6. Antimicrobial activity on synthesized AgNPs with *C. minutus* determined by MIC assay.

resistant strains. These pathogens cause the nexus of nosocomial spread in hospital settings generating clinical annoyance. Indeed, It could the biogenically synthesized AgNPs could have a future in the control of bacterial contaminants.

4. Conclusions

Cyanobacteria receive a great attention in therapeutic field. Herein, cyanobacterial samples were collected from newly developed meeting point of sea and a rivulet (brackish water); and the isolated samples were studied, based on the dominant genus, *Chroococcus* sp., which were *C. pallidus*, *C. indicus*, *C. montanus*, *C. micrococcus*, *C. minutus* and *C. schizodermaticus* as species. Particularly, *C. minutus* (strain, CRLSUM10) was cultured in BG-11, CHU#10, synthetic, low-cost media; and the growth of *C. minutus* analyzed. Moreover, the harvested biomass was used for the biosynthesis of AgNPs, whose spectral characterizations were done by UV-Vis, SEM-EDX, XRD and FTIR. Anti-microbial activities of the synthesized AgNPs had remarkable control against URTI causing bacteria. These AgNPs could be utilized as antimicrobial agents in future.

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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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.sjbs.2020.03.020>.

References

Abdel-Raouf, N., Al-Enazi, N.M., Ibraheem, I.B.M., Alharbi, R.M., Alkhulaifi, M.M., 2019. Biosynthesis of silver nanoparticles by using of the marine brown alga *Padina pavonia* and their characterization. *Saudi J. Biol. Sci.* 26, 1207–1215.

Ahmad, S., Munir, S., Zeb, N., Ullah, A., Khan, B., Ali, J., Bilal, M., Omer, M., Alamzeb, M., Salman, S.M., Ali, S., 2019. Green nanotechnology: a review on green synthesis of silver nanoparticles—an ecofriendly approach. *Int J. Nanomedicine*. 14, 5087.

Anonymous, 2018. <https://www.algalweb.net/>.

Anwer, S.S., Abdulkareem, P.M., 2014. Antibacterial activity of *Lyngbya* and *Chroococcus* species isolated from Koya (Hizooop River). *J. Life Sc.* 8, 925–930.

Baral, N., Mohapatra, S., Raiguru, B.P., Mishra, N.P., Panda, P., Nayak, S., Pandey, S.K., Kumar, P.S., Sahoo, C.R., 2019. Microwave assisted rapid and efficient synthesis of new series of chromene based 1,2,4 oxadiazole derivatives and evaluation of antibacterial activity with molecular docking investigation. *J. Heterocycl. Chem.* 56, 552–565.

Barrie, J.D., Gallacher, J.B., 1975. The significance of *Escherichia coli* in the upper respiratory tract of children under 2 years of age. *Postgrad. Med. J.* 51 (596), 373–381.

Dos, C.A.S., Seckler, M.M., Ingle, A.P., Gupta, I., Galdiero, S., Galdiero, M., Gade, A., Rai, M., 2014. Silver nanoparticles therapeutically uses, toxicity, and safety issues. *J. Pharma Sc.* 103 (7), 1931–1944.

Fernandes-Negreiros, M.M., Machado, R.I.A., Bezerra, F.L., Melo, M.C.N., Alves, M.G.C.F., Filgueira, L.G.A., Morgano, M.A., Trindade, E.S., Costa, L.S., Rocha, H.A.O., 2018.

Antibacterial, antiproliferative, and immunomodulatory activity of silver nanoparticles synthesized with fucans from the alga *Dictyota mertensii*. *Nanomaterials*, 8, 6.

Gademann, K., 2007. Cyanobacterial natural products for the inhibition of biofilm formation and biofouling. *Chimia Int. J. Chem.* 61, 373–377.

Ghasemi, Y., Moradian, A., Mohagheghzadeh, A., Shokravi, S., Morowvat, M.H., 2007. Antifungal and antibacterial activity of the microalgae collected from paddy fields of Iran: characterization of antimicrobial activity of *Chroococcus dispersus*. *J. Biol. Sc.* 7, 904.

Hafez, E.E., Kibei, S.S., 2013. Antimicrobial activity of nano-silver particles produced by micro algae. *J. Pure Appl. Microbiol.* 7, 35–42.

Husain, S., Sardar, M., Fatma, T., 2015. Screening of cyanobacterial extracts for synthesis of silver nanoparticles. *World J. Microbiol. Biotechnol.* 2 (1), 134–266.

Jenck, J.F., Agterberg, F., Droscher, M.J., 2004. Products and processes for a sustainable chemical industry: a review of achievements and prospects. *Green Chem.* 6, 544–556.

Karan, T., Altuner, Z., Erenler, R., 2017a. Growth and metabolite production of *Chroococcus minutus* under different temperature and light conditions. *J. New Res. Sc.* 6 (1), 47–52.

Karan, T., Kayir, O., Altuner, Z., Erenler, R., 2017b. Growth and norharmane production of *Chroococcus minutus* under various stress conditions. *Int. J. Chem. Tech.* 2 (1), 10–15.

Kaushik, B.D., 2014. Developments in cyanobacterial biofertilizer. *Proc. Indian Nat. Sci. Acad.* 80 (2), 379–388.

Komarkova, J., Jezberová, J., Komárek, O., Zapomělová, E., 2010. Variability of *Chroococcus* (Cyanobacteria) morphospecies with regard to phylogenetic relationships. *Hydrobiologia* 639 (1), 69–83.

Lekshmi, S., Vijayalakshmy, K.C., Reshma, G.S.S., Sharma, A.V., 2016. Antibacterial activity of *Chroococcus minutus* (Kützing) Nägeli isolated from Cochin estuary against selected pathogens. *Int. J. Fish. Aquat. Stud.* 4 (3), 700–703.

Masavkar, S.P., Naikwadi, A.M., 2016. Study of incidence of upper respiratory tract infections in urban and rural population. *Sch. J. Appl. Med. Sci.* 4, 2023–2026.

McGregor, G.B., Fabbro, L.D., Lobbeiger, J.S., 2007. Freshwater planktic Chroococcales (Cyanoprokaryota) from North-Eastern Australia: a morphological evaluation. *Nova Hedwigia* 84, 299–331.

Mohan, N., Rao, H.P., Kumar, R.R., Sivasubramanian, V., 2010. Mass Cultivation of *Chroococcus turgidus* and *Oscillatoria* sp. and effective harvesting of biomass by low-cost methods. *Nat. Proc.* <https://doi.org/10.1038/npre.2010.4331.1>.

Munir, M., Qureshi, R., Ilyas, M., Munazir, M., Leghari, M.K., 2016. Systematics of *Chroococcus* from Pakistan. *Pak. J. Bot.* 48 (1), 255–262.

Nayak, N., Padhy, R.N., Singh, P.K., 2015. Evaluation of antibacterial and antioxidant efficacy of the Fern *Azolla caroliniana* symbiotic with the cyanobacterium *Anabaena azollae*. *Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci.* 85 (2), 555–569.

Sahoo, C.R., Paidsetty, S.K., Padhy, R.N., 2019a. Norharmane as a potential chemical entity for development of anticancer drugs. *Eur. J. Med. Chem.* 162, 752–764.

Sahoo, C.R., Paidsetty, S.K., Padhy, R.N., 2019b. Norrostocine congeners as potential anticancer drugs: an overview. *Drug Dev. Res.* 80, 878–892.

Sahoo, C.R., Paidsetty, S.K., Dehury, B., Padhy, R.N., 2019c. Molecular dynamics and computational study of Mannichbased coumarin derivatives: potent tyrosine kinase inhibitors. *J. Biomol. Struct. Dyn.* <https://doi.org/10.1080/07391102.2019.1701554>.

Singh, S., Kate, B.N., Banerjee, U.C., 2005. Bioactive compounds from cyanobacteria and microalgae: an overview. *Crit. Rev. Biotechnol.* 25 (3), 73–95.

Soltani, N., Khavari-Nejad, R.A., Tabatabaei, M.Y., Shokravi, S., Fernandez-Valiente, E., 2005. Screening of soil cyanobacteria for antifungal and antibacterial activity. *Pharm. Biol.* 43 (5), 455–459.

Sompong, U., Anuntalabhochai, S., Cutler, R.W., Castenholz, R.W., Peerapornpisal, Y., 2008. Morphological and phylogenetic diversity of cyanobacterial populations in six hot springs of Thailand. *Sci. Asia* 34, 153–162.

Swain, S.S., Paidsetty, S.K., Dehury, B., Sahoo, J., Vedithi, S.C., Mahapatra, N., Hussain, T., Padhy, R.N., 2018. Molecular docking and simulation study for synthesis of alternative dapsone derivative as a newer antileprosy drug in multidrug therapy. *J. Cell. Biochem.* 119 (12), 9838–9985.

Thiel, T., Bramble, J., Rogers, S., 1989. Optimum conditions for growth of cyanobacteria on solid media. *FEMS Microbiol. Lett.* 61, 27–31.

Venkatesan, J., Kim, S.K., Shim, M.S., 2016. Antimicrobial, antioxidant, and anticancer activities of biosynthesized silver nanoparticles using marine algae *Ecklonia cava*. *Nanomaterials* 6, 235. <https://doi.org/10.3390/nano6120235>.

Vijayakumar, S., Menakha, M., 2015. Pharmaceutical applications of cyanobacteria—A review. *J. Acute Med.* 5, 15–23.

Volk, R.B., Furkert, F.H., 2006. Antialgal, antibacterial and antifungal activity of two metabolites produced and excreted by cyanobacteria during growth. *Microbiol. Res.* 161 (2), 180–186.

Vos, T., Allen, C., Arora, M., Barber, R.M., Bhutta, Z.A., Brown, A., Carter, A., Casey, D. C., Charlson, F.J., Chen, A.Z., Coggeshall, M., 2016. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the global burden of disease study 2015. *The Lancet*. 388, 1545–1602.

Wood, S.A., Rhodes, L., Smith, K., Lengline, F., Ponikla, K., Pochon, X., 2017. Phylogenetic characterization of marine *Chroococcus*-like (Cyanobacteria) strains from the Pacific region. *N. Z. J. Bot.* 55 (1), 5–13.