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Utilization of 30 Cyanobacterial extracts for the Synthesis, Screening and Optimization of AuNPs: A Promising Approach for Green Chemistry

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ABSTRACT: Gold nanoparticles (AuNPs) became a wide area of research, due to their unique chemical and physical features they are widely used in biological applications. The challenges of the study was to synthesized less toxic ecofriendly nonhazardous biocompatible nanoparticles. Considerable increasing demand of such AuNPs in different fields, especially in nanomedicines, there is an urgent need to maintain constant supply of top quality biogenic AuNPs. Material methods: 30 cyanobacterial extracts were screened for AuNPs synthesis based on the minimum reduction time and size of NPs. Best strain optimized with set controlled conditions of 1mM $HAuCl_4$, 45 ml of extract volume, pH 6.5, and 60°C. And characterized by UV-Visible, FTIR and AFM characterized. Results: Purple to ruby red of reaction mixture is visual indication of AuNPs, further confirmed by UV–Vis spectroscopy and SEM, FTIR and AFM. During screening *Phormidium* sp. synthesized spherical smallest AuNPs (08-23 nm) within 35 minutes. Optimization resulted in reduction in size from 08-23 nm to 04-07 nm (SEM) and time 35 to 25 min.

Keywords: Cyanobacteria, Gold nanoparticles (AuNPs); Screening, Optimization, FTIR, AFM.

INTRODUCTION

In present era nanoscience and nanotechnology became the advanced topic of research due to potential applications in various fields. Green route for nanoparticles synthesis emerged as comparatively much better, safe, less toxic techniques for nanoparticles biosynthesis than chemical synthesis. Green synthesis of AuNPs include cell free extracts of biological entities like plants (Khan *et al.,* 2018), algae (Ghodake and Lee 2011; Oza *et al.,* 2012) fungi (Mishra *et al.,* 2012) bacteria (Pourali *et al.,* 2017) and cyanobacteria (Parial *et al.,* 2012). Cyanobacteria have attracted attention of researchers due to their easy maintenance and high biomass production in low cost and less time (Bakir *et al.,* 2018). Cyanobacteria and algae serve as natural sources for the synthesis of selenium, silver, zinc, titanium, platinum, and palladium nanoparticles (Afzal *et al.,* 2019; Zaki *et al.,* 2022; Ahamad *et al.,* 2022; Aziz *et al*., 2015; Asif *et al*., 2021; Siddiqui *et al.,* 2022; Brayner *et al.,* 2007; Cookson, 2012).

The AuNPs gained special interest over the last few decades because of their distinctive chemical, physical properties and applications as antibacterial, antifungal (Khan *et al*., 2018) and anti-inflammatory agent (de Araújo Júnioret *et al.,* 2017). Besides, AuNPs were also used in drug delivery (Li *et al.,* 2022; Karagoz *et al*., 2014) cancer and photo thermal therapy (Vines *et al.,* 2019), bio-conjugates formation (Zhang *et al.,* 2008-a & b) optical-nanosensors (Kneipp *et al.,* 2010; Anh *et al.,* 2022) catalytic degradation (Umamaheswari *et al*., 2018) bio-imaging (Hutter *et al.,* 2011; Si *et al.,* 2021) etc.

The applications of AuNPs in different fields especially in nanomedicines needs constant supply of enough biogenic AuNPs. Therefore, during present investigation as attempt was made to add novel cyanobacterial derived AuNPs this included screening of 30 different cyanobacterial extracts for AuNPs synthesis. Optimization of condition for synthesis of smallest AuNPs in least possible time and examining the best AuNPs for physiochemical characterization and bioactivity (all data not shown here already communicated).

MATERIALS AND METHODS

Chemicals. The chemicals used in this study were of analytical `grade and were purchased from Sigma (St. Louis, USA) and Hi-Media. The buffers, reagents and extracts were prepared in distilled water. All the glass wares were washed with double distilled water and air-dried.

Microbial cultures and growth conditions. Cyanobacterial strains were procured from CFTRI, Mysore; IARI, New Delhi; NFMC, Tiruchirappalli, India. Axenic cultures were routinely grown in 500 ml

Erlenmeyer flasks aseptically in BG-11 liquid medium (with and without sodium nitrate) in culture room at 27 ± 2°C and illuminated with cool white Sylvania 40W T12 fluorescent lamps having a lux intensity of 2000 ± 200 for 12 h light/dark cycles (Stanier *et al.,* 1971) *Spirulina* and *Arthrospira* strains were grown in Zarrouk's media (Zarrouk 1966). Growth was observed using aUV-Vis spectrophotometer (Labtronics, LT 2900) by recording the absorbance at 750 nm for period of 30 days.

Preparation of cyanobacterial extract. Cyanobacterial strains were harvested in the mid-exponential growth phase for extract preparation using the modified protocol of MubarakAli. (2011) 2.5 g lyophilized biomass was suspended in 100 mldouble distilled water (ddH_2O) and homogenized for 5 min to ensure the complete breakage of cells. The resulting extract were kept in water bath for 25 min at 65 ℃ and then centrifuged at 6500 rpm for 10 min. The supernatant was collected and filtered through Whatman No.1 filter paper to remove cell debris. The filtrate was collected in 250 ml Erlenmeyer flask and was reconstituted to final volume of1L ddH_2O. The resulting extract was stored in refrigerator at 4 ℃ for further use.

Biosynthesis and optimization of gold nanoparticles. For the extracellular synthesis of AuNPs, in 60 ml of cell free extracts added 40 ml of 0.6 mM $HAuCl_4.3H_2O$. The cell free extracts and chloroauric acid solution alone were taken separately as positive and negative controls. The pH of reaction mixture was adjusted to 5.5. The flasks were incubated at 40°C for 5 h. The color change from purple to ruby red indicated the synthesis of AuNPs that were harvested by centrifugation at 12,000 rpm for 20 min at 4 ℃. The nanoparticles were thoroughly washed with distilled water and ethanol successively to remove impurities. The resulting nanoparticles were lyophilized and stored at -20 ℃ for further analysis. The biosynthesized AuNPs were scanned by–Vis's spectrophotometry in 300–700 nm range (Labtronics LT 2900) and SEM.

Optimization of biosynthesized AuNPs. Best cyanobacterial strain *Phormidium* sp. NCCU-104 was optimized for reaction conditions for cell extract volume, chloroauric acid, conc., pH., temperature, and reaction time for synthesis of smallest AuNPs in minimum time.

Physical characterization of *Phormidium* sp. derived AuNPs. UV-visible spectroscopy the biosynthesized AuNPs were scanned by–Vis's spectrophotometry in 300–700 nm range (Labtronics LT 2900). FTIR was done to know the functional group present in AuNPs. They were finely ground and dispersed in pellets of KBr (Sigma) for FTIR spectrum in 500–4000 cm^{-1} range at a resolution of 4 cm^{-1} on Varian 7000 FTIR system (PerkinElmer, US). Atomic force microscopy (AFM) was done to found the 2D AND 3D image and size of the biosynthesized AuNPs for this drop of (1µmg/ml AuNPs solution) AuNPs placed on glass slide and dried under laminar flow and observed slide by AFM (Model Ntegra Prima AFM, NT-MDT, Russia).

RESULTS AND DISCUSSION

Screening. In present study, 30 cyanobacterial strains were screened for their potential to synthesize AuNPs (Fig. 1). Color change from purple to ruby red indicated the AuNPs synthesis (Fig. 3a). During the UV-Visible spectra (300-700 nm) scanning peak for AuNPs was observed at 520-535 nm [Fig. 2(a-e)]. All the strains exhibited potential to synthesize AuNPs but with difference in their morphology, size, and reduction time. Time taken for AuNPs synthesis ranged from 35 min to 5 hours and their size ranged from 08-274 nm (Table 1). SEM analysis depicted spherical, hexagonal, triangular, and cubic shaped AuNPs [Table 1 and Fig. 3 (a,b].

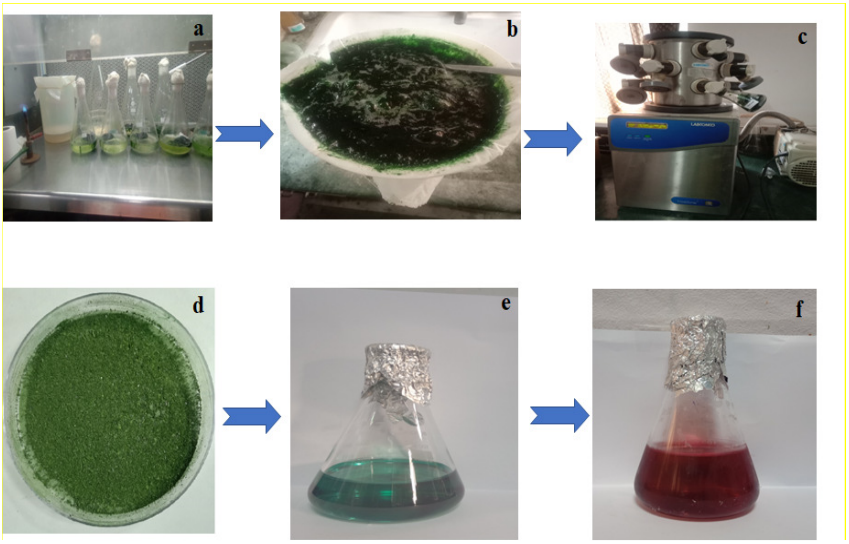

Fig. 1. Different steps of AuNPs synthesis using *Phormidium* cell extract (a) Cultures of *Phormidium* sp. *NCCU-104* (b) Biomass Harvested (c) Lyophilization of Biomass (d); powder preparation of biomass of lyophilized biomass (e); cell Extract preparation (f); AuNPs synthesis.

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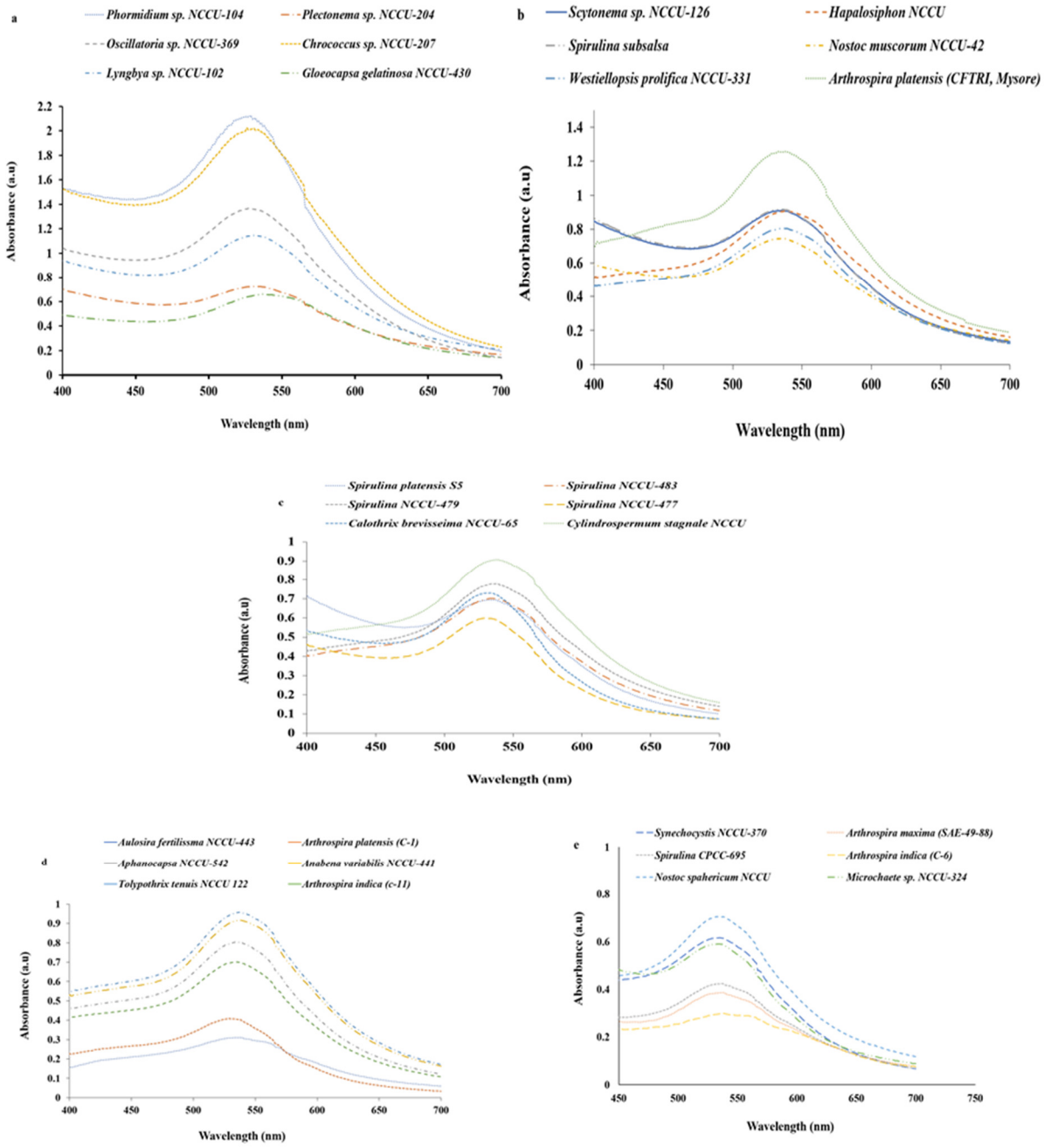

Fig. 2 (a-e).UV-Vis. Scanning of AuNPs synthesized by *Phormidium* sp. NCCU-104, *Plectonema* sp. NCCU-204, *Oscillatoria* sp. NCCU-369, *Chrococcus* sp. NCCU-207, *Lyngbya* sp. NCCU-102, *Gloeocapsa gelatinosa* NCCU-430 **(b)** *Scytonema* sp. NCCU-126, *Hapalosiphon* NCCU, *Spirulina subsalsa, Nostoc muscorum* NCCU-42, *Westiellopsis prolifica* NCCU-33 and *Arthrospira platensis* **(c)** *Spirulina platensis* S5, *Spirulina* NCCU-483, *Spirulina* NCCU-479, *Spirulina* NCCU-477, *Calothrix brevisseima* NCCU-65, *Cylindrospermum stagnale* NCCU ***(d)*** *Aulosira fertilissma* NCCU-443, *Arthrospira platensis* (C-1), *Aphanocapsa* NCCU-542, *Anabena variabilis* NCCU-441, *Tolypothrix tenuis* NCCU 122 and *Arthrospira indica* (C-11) **(e)***Synechocystis* NCCU-370, *Arthrospira maxima* (SAE-49-88), *Nostoc spahericum* NCCU, *Spirulina* CPCC-695, *Arthrospira indica* (C-6) and *Microchaete sp.* NCCU-324.

Fig. 3a. Change of color after AuNPs synthesis from different 30 cyanobacterial strains.

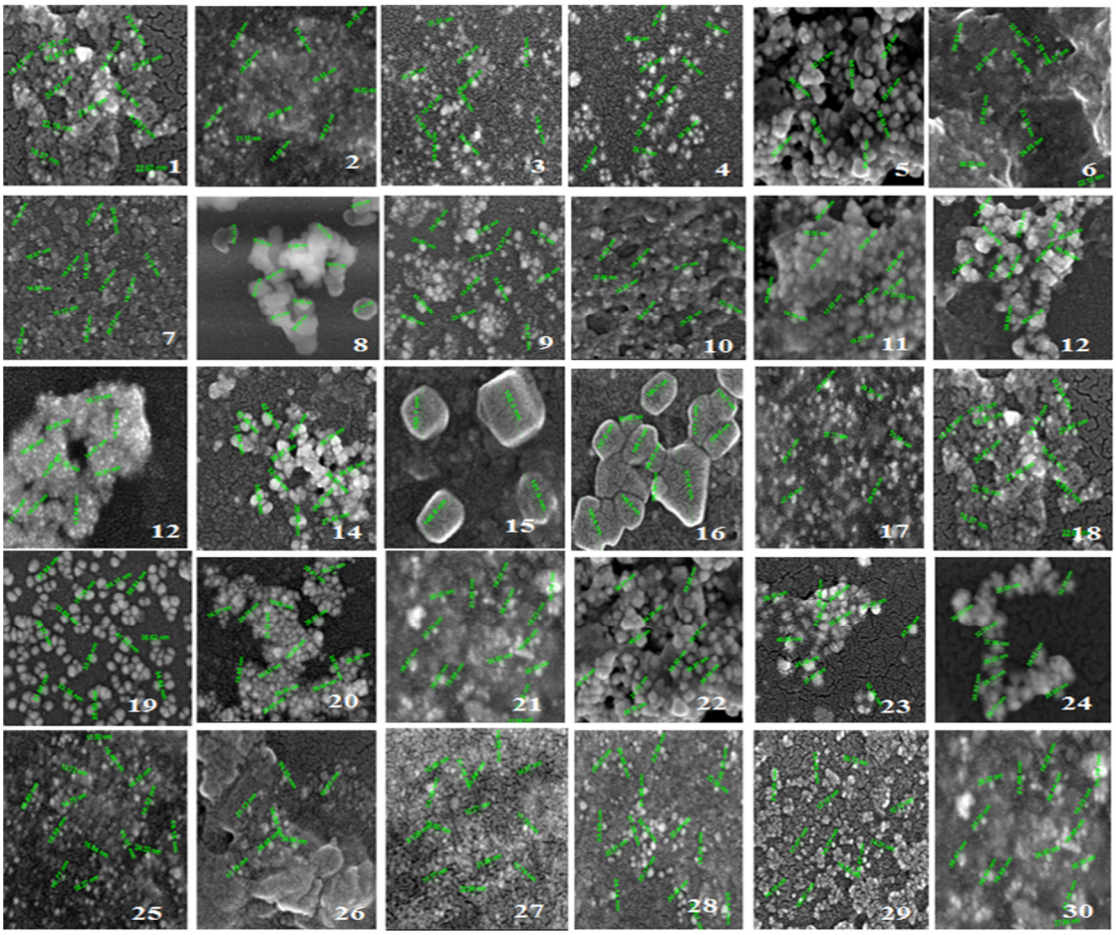

Fig. 3 (b). SEM images of biosynthesized AuNPs from different 30 cyanobacterial strains i.e. (1) *Phormidium* sp. NCCU-104, (2) *Plectonema* sp. NCCU-204, (3) *Oscillatoria* sp. NCCU-369,(4) *Chrococcus* sp. NCCU-207, (5) *Lyngbya* sp. NCCU-102, (6) *Gloeocapsa gelatinosa* NCCU-430(7) *Scytonema* sp. NCCU-126, (8) *Hapalosiphon* NCCU, (9) *Spirulina subsalsa,* (10) *Nostoc muscorum* NCCU-42, (11) *Westiellopsis prolifica* NCCU-33 (12) *Arthrospira platensis,* (13) *Spirulina platensis* S5, (14) *Spirulina* NCCU-483, (15) *Spirulina* NCCU-479, (16) *Spirulina* NCCU-477, (17) *Calothrix brevisseima* NCCU-65, (18) *Cylindrospermum stagnale* NCCU (19) *Aulosira fertilissma* NCCU-443, (20) *Arthrospira platensis* (C-1), (21) *Aphanocapsa* NCCU-542, (22) *Anabena variabilis* NCCU-441, (23) *Tolypothrix tenuis* NCCU 122 (24) *Arthrospira indica* (C-11), (25) *Synechocystis* NCCU-370, (26) *Arthrospira maxima* (SAE-49-88), (27) *Nostoc spahericum* NCCU, (28) *Spirulina* CPCC-695, (29) *Arthrospira indica* (C-6), (30) *Microchaete* sp. NCCU-324.

Table 1: SEM and UV based screening of cyanobacterial for AuNPs synthesis.

Sr. No.	Cyanobacterial strain	Peaks (nm)	Shape	Size (nm)	Time taken (minutes)
1.	*Phormidium* sp. NCCU-104*	520	Spherical	08-23	35
2.	*Plectonema* sp. NCCU-204	530	Spherical	16-24	50
3.	*Oscillatoria* sp. NCCU-369	524	Spherical	12-36	110
4.	*Chrococcus* sp. NCCU-207	524	Spherical	16-35	45
5.	*Lyngbya* sp. NCCU-102	528	Spherical	28-39	65
6.	*Gloeocapsa gelatinosa* NCCU-430	534	Spherical	14-31	180
7.	*Scytonema* sp. NCCU-126	530	Spherical	10-26	50
8.	*Hapalosiphon* NCCU	532	Spherical	41-87	135
9.	*Spirulina subsalsa*	528	Spherical	14-45	40
10.	*Nostoc muscorum* NCCU-42	532	Spherical	12-41	140
11.	*Westiellopsis prolifica* NCCU-331	534	Spherical	10-40	60
12.	*Arthrospira platensis* (CFTRI, Mysore)	529	Spherical	29-49	90
13.	*Spirulina platensis* S5	530	Spherical	10-18	60
14.	*Spirulina* NCCU-483	528	Spherical	26-42	260
15.	*Spirulina* NCCU-479	530	Square, cubic	146-192	220
16.	*Spirulina* NCCU-477	526	Triangular-hexagonal	78-274	180
17.	*Calothrix brevisseima* NCCU-65	526	Spherical	24-33	57
18.	*Cylindrospermum stagnale* NCCU	530	Spherical	13-22	60
19.	*Aulosira fertilissma* NCCU-443	530	Spherical	25-41	180
20.	*Arthrospira platensis* (C-1)	526	Spherical	15-38	240
21.	*Aphanocapsa* NCCU-542	530	Spherical	18-48	55
22.	*Anabena variabilis* NCCU-441	534	Spherical	19-38	75
23.	*Tolypothrix tenuis* NCCU 122	530	Spherical	24-45	70
24.	*Arthrospira indica* (C-11)	530	Spherical	25-59	120
25.	*Synechocystis* NCCU-370	530	Spherical	12-24	75
26.	*Arthrospira maxima* (SAE-49-88)	530	Spherical	10-26	245
27.	*Nostoc spahericum* NCCU	530	Spherical	09-22	192
28.	*Spirulina* CPCC-695	534	Spherical	18-35	120
29.	*Arthrospira indica* (C-6)	535	Spherical	12-23	60
30.	*Microchaete* sp. NCCU-324	530	Spherical	19-38	280

In our results smallest sized (08-23 nm), spherical AuNPs were obtained with *Phormidium* sp. extract in 35 minutes. Younis *et al.* (2019) found purple color AuNPs with diameter of 41.7 ± 0.2 nm and 80 ± 30 nm for *Lyngbya majuscula* and *Cyanothece* sp. respectively Rajeshkumar *et al.* (2013) synthesized pinkish ruby red AuNPs with average 60 nm size after 24 hours of incubation from brown algae *Padina tetrastromatica and Turbinaria conoide.* Singaravelu *et al.* (2007) found ruby red, 8-12 nm AuNPs from algae *Sargassum swartzii* after 15 hours. AuNPs are also synthesized from higher plants e.g.,19- 24 nm ruby red, spherical AuNPs from the higher plant *Acer pentapomicum* (Khan *et al.,* 2018).

Optimization for AuNPs synthesis. After screening the best cyanobacteria (*Phormidium* sp.) was optimized for reaction conditions (cell extract volume, chloroauric acid conc., pH, temperature and reaction time). Highest peak was observed with 1m $MHAuCl_4$ (Fig. 4a). Further increase in $HAuCl_4$ concentration resulted in flattering of peak probably by aggregations of nanoparticles. Princy *et al.* (2018) also found best AuNPs synthesis with 1 mM $HAuCl_4$ with *Padina tetrastromatica* extract. Any increase or decrease in volume of extract directly affected the AuNPs biosynthesis. The best result was found with 45 ml of cell extract (Fig.4b). Further increase in the volume (60 ml and 75 ml) resulted in sudden decrease in the peak intensity along with appearance of flattening of the peak. Another most important factor of AuNPs synthesis is pH, because it directly affects the shape and size of the AuNPs (Busch *et al.,* 2019). Best AuNPs synthesis took place at pH of 6.5 (Fig. 4c). Zabetakis *et al.* (2012) also observed chemically synthesized AuNPs at nearly pH 6.5. The absorption intensity the AuNPs peak increased with temperature and was highest at 60°C, [Fig. 4(d)]. Further temperature increases decreased thesharpness of the peak and resulted in peak flattening, probably due to aggregation of the AuNPs. Wongyai *et al.* (2020) also noticed good AuNPs synthesis at 60 °C from plant extract of *Cryptolepis buchanani*. Khan *et al.* (2018)reported temperature from 70 to 100 °C, almost all the synthesized nanoparticles were degraded. In our findings mostly AuNPs were synthesized within 3 hours (Table 1). Ahmad *et al.* (2016) studied the effect of time on AuNPs synthesis, shape, size and stability.

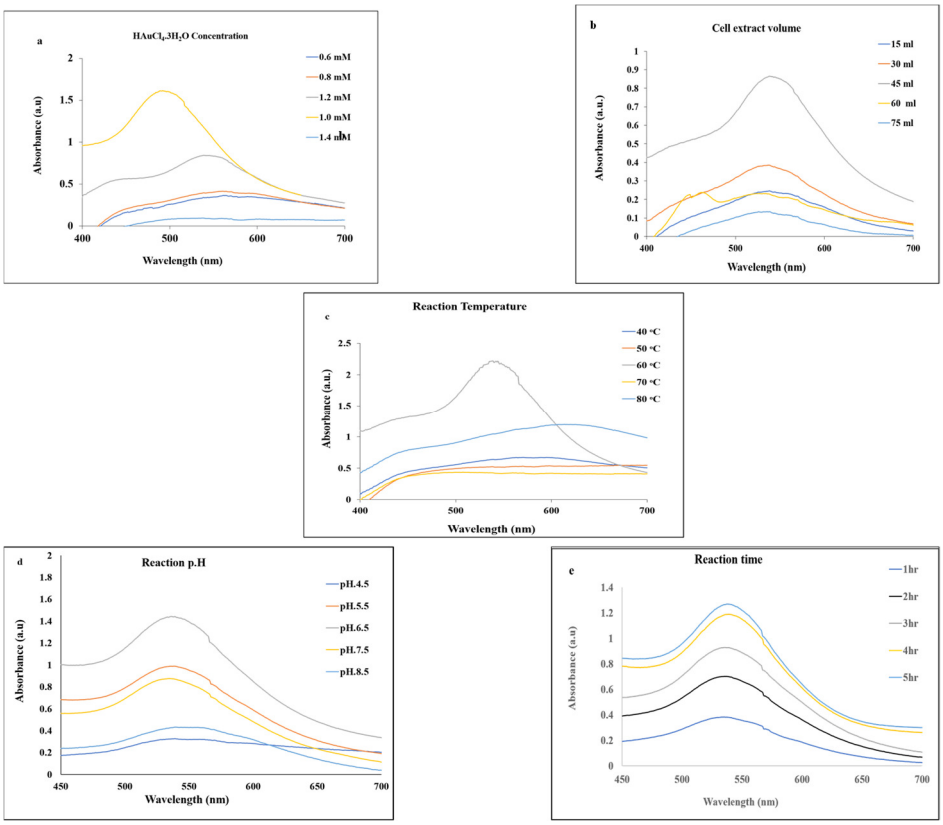

Fig. 4 (a-e). Optimization reaction conditions for AuNPs synthesis, (a) $HAuCl_4$ concentration, (b) *Phormidium* cell extract volume, (c) reaction temperature (d) reaction pH (e) reaction time

Physical characterization of *Phormidium* derived AuNPs. The bioreduction of the Au (III) ions to Au was monitored by UV–Vis spectra at wavelength 400-700 nm and 1cm resolution path length. A single SPR peak band at 520 nm was observed inAuNPs synthesized from *Phormidium* sp. (Fig. 5a). AuNPs from *Lyngbya* also exhibited peak at 529 nm (Parial and Pal 2014). No similar peaks were not observed in chloroauric acid/ salt precursor (Fig. 5b) and *Phormidium* sp. extract (Fig. 5c). And figure d showed color of extract (green), salt (yellow) and biosynthesized AuNPs (ruby red) position of vials from left to right. Similar results was found by Parial and Pal (2015); Bakir *et al.* 2018.

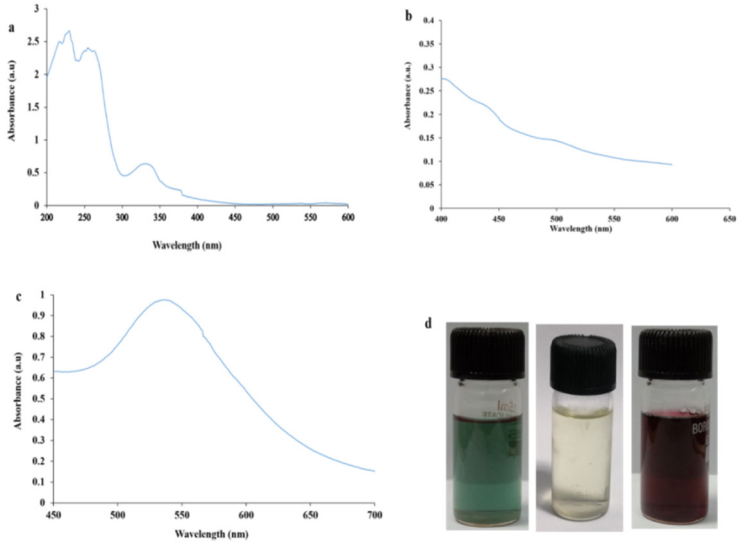

Fig. 5. Characterization of synthesized AuNPs derived from *Phormidium* sp. by (a) UV-visible spectra of (a) *Phormidium* sp. extract, (b) Chloroauric acid solution (c) *Phormidium* sp. derived AuNPs. (d) *Phormidium* sp. extract (green), Chloroauric acid solution (yellow) and synthesized AuNPs (red) from left to right.

Fourier Transform Infrared Spectrometer (FTIR): FTIR analysis. The functional groups were analyzed by FTIR spectra, presence of different peaks show the presence of different functional groups which predicted that possible reducing agents responsible for synthesis (Hamida *et al.,* 2020) Peak at 3778 cm^{-1} represents (O-H) stretching in intermolecular bonding along with N-H stretching for aliphatic primary amines. Peaks at 2864 to 2926 cm^{-1} showed the presence of (CH_3, CH_2, CH and aldehyde C-H) and (N-H) stretching for amine salts. Whereas Peak at 1600 cm^{-1} showed disubstituted alkenes (C=C) and amines (N-H). Aromatic nitro (N-O) compound was shown by the peak at 1520 cm^{-1}. Similarly alkane, amine, amide shows the peaks at 1457, 1245 and 1061 cm^{-1} respectively. Peak at 1457 cm^{-1} showed alkane (C-H) methyl group. Peaks at 1245 and 1061 cm^{-1} (C-N) sowed the presence of amine and (C-O) stretching for alkyl ether respectively. Proteins possibly act as AuNPs capping agents as they have stronger ability to bind with the metals. Near about Similar peaks were also seen by khan *et al.,* (2018); Geetha *et al.,* (2013) in *Acer pentapomicum* and *Couroupita guianensis* respectively. Younis *et al.* (2019) also found FTIR spectra of different functional groups involve in biosynthesis of AuNPs by *Cyanothece* sp. extract. Zhao et. al., (2021) interpreted and confirmed bioreduction of AuNPs present in extract of *Dendrobium officinale* (medicinal plant) reason of bioreduction of AuNPs.

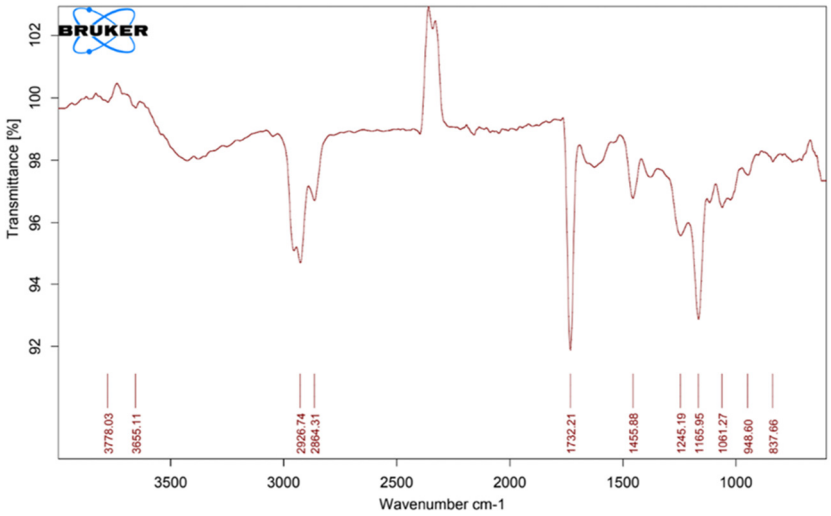

Fig. 6.

Atomic force spectroscopy (AFM). During AFM analysis 2D and 3D view of the sample surface over a 1-1 µm scan with uniform height distribution was taken. The size of AuNPs obtained from tip-corrected AFM measurements was in the range of 04 - 07 nm (Fig. 7) and the spherical shape of AuNPs was determined. The chemically synthesized spherical of 20 nm, 30 nm and 40 nm AuNPs were observed by the Zakaria *et al.* (2013). Biologically synthesized 10 nm AuNPs synthesized by the Zhao *et al.* (2021) from *Dendrobium officinale*.

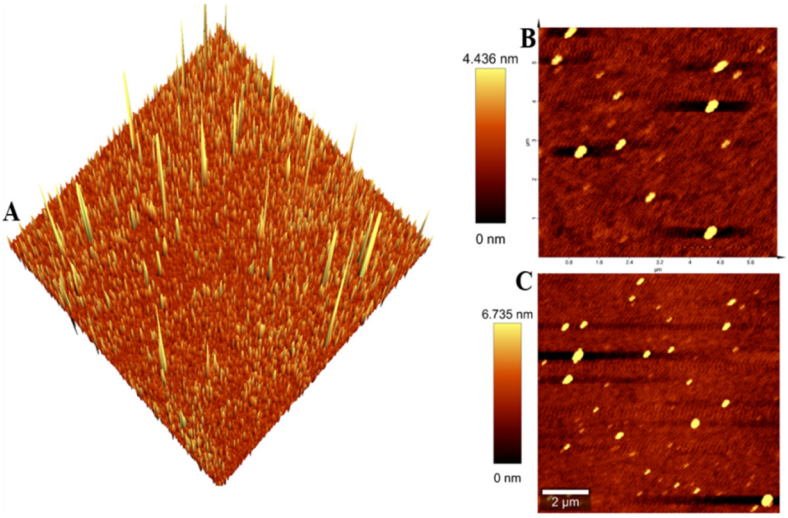

Fig. 7. 3D and 2D view Atomic force micrographs (AFM) of biosynthesized AuNPs from *Phormidium* sp.(A) 3D view (B & C) 2D view showing size 4.436 nm and 6.735 nm size of nanoparticles.

CONCLUSIONS

Through present study success was achieved in adding cyanobacteria as a novel source for biogenic AuNPs. All cell extracts (30 cyanobacteria) exhibited color change of reaction mixture from purple to ruby red indicating their potential AuNPs synthesis. *Phormidium* synthesized smallest 08-23 nm nanoparticles within 35 minutes and appeared as best the strain. Optimization of reaction condition for AuNPs synthesis resulted in reduction of size (08-23 nm to 04-07 nm) and time (35 min to 25 min), that were characterized by UV visible spectroscopy, FTIR and AFM analysis.

FUTURE SCOPE

The synthesized biogenic AuNPs have great applications in the field of agriculture (as nanofertlizers, Nanopesticides) and in field of nanomedicines. It was observed by many researchers that accelerated seed germination and enhance overall production of plants treated with gold nanoparticles, so AuNPs could be used in agricultural purposes for better yield.

Authors contribution: S: Investigation, Methodology, analysis, Writing - original draft, Visualization. NA^1&NA^2 formal analysis TF: and NA^3 analyze the data, conceptualization, Resources, Supervision, Data curation. All authors read and approved the final manuscript. PS and RK funds supporting. (NA^1 =Nida Asif, NA^2 = Nafe Aziz, NA^3 =Nadeem Ahmad).

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Conflict of Interest. None.

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