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# RESEARCH ARTICLE

## SYNTHESIS OF POLYSACCHARIDE MEDIATED COPPER NANOPARTICLES USING *SHOUCHELLA CLAUSII* AND STUDY OF ITS *IN VITRO* ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES

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## ABSTRACT

**Background:** Polysaccharides from *Shouchella clausii* was characterized and the production was optimized with different variables using Box–Behnken experimental design. FTIR and $^{1}$H NMR analysis revealed the presence of functional groups corresponding to carbohydrates, proteins, and sulphates. **Result:** The polysaccharide-based nanoparticle was synthesized using $CuSo_4$ and it was primarily screened for antibacterial, antioxidant and anticancer activity. In the present study, the antioxidant potential of a polysaccharide mediated synthesized CuNPs was estimated by DPPH and reducing power assay which showed 35.95 % at 1 mg/mL and $A_{700}$ 0.495 at 1 mg/mL respectively. **Conclusion:** The present study makes an understanding and its possibility of potential uses.



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# INTRODUCTION

The bacterial polysaccharides have gained more attention because of their bioactive properties. Polysaccharides are "sustainable" and "biobased" materials. The low degree of polymerization and degree of deacetylation, confers the antimicrobial activity of the bacterial polysaccharides hence it readily penetrates into the cell membrane of the bacteria and interrupts the process of replication (Poli *et al*. 2011.,Nwodo *et al*. 2012). These astounding features leads to the development of unique type of polymer based metallic nanoparticles. (Prakasham *et al*., 2012, Mehta *et al*., 2014, Ramalingam *et al*., 2014). The bacteria produced an adequate level of exopolysaccharide (Degeest B *et al*.,2001) to protect from environmental insults like desiccation, predation, and antagonistic substances (Sutherland IW 2001). Various factors like temperature, pH, mineral requirements, carbon and nitrogen sources influence the bacterial growth and exopolymer production. The polysaccharides promoted the bacterial cells to bind on the surfaces of animate and inanimate materials and aggregate to form a biofilm, and in the uptake of nutrients (Holmstrom C and Kjelleberg S 1999, Nichols *et al*., 2005). Bacterial polysaccharides are well-recognized metal chelator, wherein metal ions are reduced to nanoparticles through series of metal transformation on polymer matrix (Morsy *et al*., 2014). The presence of different functional groups bacterial exopolymer involve in metal reduction and stabilization of colloidal state nanoparticles. In this study, polysaccharide-based Copper nanoparticle has been synthesized and then evaluated the effectiveness for various properties. The existence of chemical inertness, biocompatibility, oxidation resistance and wide spectrum of antimicrobial activity against diverse range of bacteria and fungi. Nanoparticles (NPs) has attracted increasing interest and being the most important candidates of choice among the nano particles (Zhang *et al*., 2018, Siddiqi *et al*., 2018). The term antioxidant represents the ability to inhibit the process of oxidation (Burlakova *et al*., 1975). The antioxidants are substances that delays or inhibits the oxidation of the substrate and it suppresses oxidation by inhibiting the formation of free radicals by scavenging radicals (Gutteridge, 1994). Therefore, the substrate with antioxidants and free radical scavenging properties may be significant in the prevention and therapeutics of diseases. The free radicals predominantly reactive oxygen species (ROS) are produced during metabolism, or sometimes the immune system produced free radicals to neutralize the antigen. Free radicals contain one or more unpaired electrons formed during metabolic stress, exposure to pollution, radiation, herbicides, chemicals, and excessive use of certain drugs. It causes oxidative damages to

proteins, DNA, and lipids. Though, natural defense mechanisms block the damaging activity of free radicals or ROS (Halliwell *et al*., 1995), however, prolonged exposure to such compounds may increase the number of free radicals in the body and cause oxidative damages to the cell (Tseng *et al*., 1997). In general metal NPs were synthesized by using plant extracts (Ghoreishi, S.M *et al*., 2011), fungi (Ronavari *et al*., 2018), bacteria, and enzymes (Brayner *et al*., 2017). It was demonstrated that the metabolites polyphenols, sugars, proteins, and vitamins of plants, bacteria and fungi are responsible for the reduction of metal ions to form NPs (Moyo *et al*., 2012). The AgNPs possess the properties of antibacterial (Shanmuganathan *et al*., 2020), antioxidant (Mohanta *et al*., 2017), anticancer activity (Ratan *et al*., 2020).

# METHODS

**Isolation and Characterization of exopolymer producing bacteria:** The exopolysaccharide producing bacterial strain was isolated from rhizosphere soil, characterized and identified by 16S rRNA sequencing method. The isolated strain was identified on the basis of the comparison with the most similar sequences of BLAST similarity search tool (Parthiban Karuppiah *et al*., 2015).

**Box-Behnken model of optimizing exopolymer production:** The bacterial exopolysaccharide production was optimized by selecting the appropriate carbon and nitrogen sources and providing different concentrations (0.5 % to 2.5 %) of carbon (glucose, sucrose, lactose, and galactose) and nitrogen (peptone, yeast extract, ammonium chloride, and ammonium sulphate separately in the basal salt medium. Subsequently, the medium was optimized by Box-Behnken model using three variables (salt concentration, pH and growth temperature). The exopolysaccharide was produced by using 2 mL of 24 h culture of isolated bacterial strain as inoculum and allowed to grow for 72 h in a rotary shaker at 110 rpm. The experimental design, statistical and graphical analyses of the data obtained were performed using 'Design Expert' software (version 9, Stat-Ease, Inc., Minneapolis, USA).

**Characterization of exopolymer:** The exopolysaccharide was extracted (Parthiban Karuppiah *et al*., 2015) and the total sugar (Dubois *et al*., 1956), protein (Lowry *et al*., 1951), uronic acid (Filisetti-Cozzzi *et al*., 1991), and sulphur (Dodgson 1961) were determined spectrophotometrically. The exopolysaccharide was analysed by high performance liquid chromatography (Filomena Freitas *et al*., 2009), FT-IR and the thermal property of bacterial exopolysaccharide was analysed in Differential scanning calorimeter (DSC) (Mettler Toledo DSC 822e) in the range of 40-450 °C (10 °C/min) under nitrogen atmosphere (Mishra *et al*., 2011).

**Synthesis and Characterization of polymer based CuNPs:** The polysaccharide mediated Copper nanoparticles were synthesized by adding 1 ml of polysaccharide suspension to the 1mM solution of 99 ml of aqueous $CuSO_4$.5 $H_2SO_4$. The reaction mixture was incubated in the dark for different time intervals. The appearance of deep brown color after the incubation was considered as an indication for the synthesis of Copper nanoparticles and it was monitored by ultraviolet-visible (UV-Vis) absorption spectrophotometer. The non reacted $CuSO_4$ were removed by centrifugation followed by washing with water. The obtained CuNPs were further purified by 12-kD cutoff dialysis bag by suspending in 100-ml of 2-(4-(2- hydroxyethyl)piperazin-1-yl) ethane sulfonic acid buffer (pH 7.4, 20 mM) and amending with sucrose for maintaining the solution density of 2.5 g/ml for 12 h. The dialysed components were centrifuged at 10,000 rpm for 1 h at 4°C to collect the supernatant and pellet. The size and shape of the Copper nanoparticles were recorded in SEM.

**Applications of bacterial exopolymer Antibacterial Activity:** Antibacterial activity of polysaccharide based nanoparticle was determined using agar well diffusion method. Bacterial pathogens such as *E.coli, Shigella dysenteriae, Staphylococci aureus,* and *streptococci pyogens,* were used as test strain. The test strains were prepared by inoculating 24 hrs culture in 50 mL of nutrient broth and incubate at 37 °C for 6 hrs. After 6 hrs, using sterile cotton swab the test strains were aseptically smeared over the Muller Hinton agar media using sterile cork borer wells were created on the Muller Hinton agar plates. Different concentration (5 to 20 µg/ml) of synthesized nanoparticles were loaded in each well and incubated at 35 ± 2°C overnight. After incubation, a zone of inhibition of bacterial growth that appeared around the well as a result of the antimicrobial action of CuNPs was recorded (Rauf *et al.,* 2017).

**Anti oxidant assay**

***In vitro* DPPH free radical scavenging assay:** The scavenging activity of the DPPH free-radical was assayed according to the method of Shimada *et al.,* (1992). Briefly 1 mL of DPPH radical solution (0.1 mM) was added with 4 mL of different concentrations (50, 100, 250, 500 and 1000 µg) of polysaccharide based CuNPs. The mixture was shaken vigorously and incubated for 15 mins in the dark at room temperature. After 15 minutes, the reduction of the DPPH radical was measured by continuous monitoring of the decrease of absorption at 517 nm (Tech Comp model: UV2301). L-ascorbic acid was used as standards. The DPPH radical scavenging activity was calculated by the formula mentioned below.

$$\text{Scavenging effect (\%)} = \frac{\text{A517 of control} - \text{A517 of sample}}{\text{A517 of control}} X\ 100$$

**Reducing power assay:** The reducing power was determined by adding 1 mL of different concentration of exopolymer based CuNPs (200, 400, 600, 800 and 1000 µg/ mL) and 2.5 mL of phosphate buffer (0.2 M) (pH 6.6) with 1 % potassium ferric cyanide. After incubation at 50°C for 20 min, 2.5 mL of Trichloroacetic acid (10 %) was added and centrifuged at 3000 rpm for 10 min.

Finally, the upper layer (2 mL) was collected and mixed with 2 mL of distilled water and 0.5 mL of 0.1 % $FeCl_3$. During this reaction the CuNps donates an electron result in $Fe^{3+}$ is reduced to $Fe^{2+}$. The amount of $Fe^{2+}$ complex formed can be monitored by measuring the formation of prussian blue at 700 nm. Higher absorbance values indicate greater reducing ability (Song *et al*., 2008).

# RESULTS

**Isolation of polysaccharide producing strain:** The isolated strain appears sticky texture. The blast similarity search results of 16S rRNA sequences revealed that the exopolymer producing strain had resemblance with *Shouchella clausii* (accession no MZ128362).

**Optimization of exopolymer production:** *Shouchella clausii* produced maximum yield of exopolymer (66.56 mg $L^{-1}$), when 2.5 % of sucrose was provided as a carbon source (Table 1). Amongst different nitrogen sources *Shouchella clausii* produced 49 ± 0.15 mg $L^{-1}$ using peptone. The production rate accelerates on the basis of particular carbon to nitrogen combination. The media was prepared with sucrose and peptone in the ratio viz.,14.68:1.00, 12.23:1.00, 10.48:1.00, 9.17:1.00, 8.15:1.00, 7.34:1.00 and the production was estimated. The optimum carbon to nitrogen ratio for *Shouchella clausii* was 14.68:1.00 and produced 115.16 ± 0.28 mg $L^{-1}$ of polysaccharide in basal salt medium (Table 2). The Box-Behnken model of RSM was validated in shake flask level experiment (Table 3) and the data were statistically analyzed. The polynomial equation was derived based on the experimental factors, quadratic effect of the factors and the interactions among the factors by the input of values in equation (1). Where Y is the response (exopolymer).

**Table 1. Effects of various sugars on exopolymer production**

| Carbon source | Exopolymer production for various concentrations of sugars (mg$L^{-1}$) | | | | |
|---|---|---|---|---|---|
| | **0.50 %** | **1.00 %** | **1.50 %** | **2.00 %** | **2.50 %** |
| **Glucose** | 39.73 ± 0.11 | 45.56 ± 0.11 | 51.1 ± 0.1 | 59.7 ± 0.1 | 63.5 ± 0.05 |
| **Sucrose** | 41.13 ± 0.15 | 47.56 ± 0.15 | 54.4 ± 0.1 | 60.46 ± 0.05 | 66.56 ± 0.11 |
| **Lactose** | 39.16 ± 0.15 | 42.93 ± 0.05 | 47.6 ± 0.17 | 52.56 ± 0.05 | 57.66 ± 0.15 |
| **Galactose** | 38.6 ± 0.17 | 41.45 ± 0.05 | 45.43 ± 0.11 | 49.7 ± 0.17 | 53.16 ± 0.1 |

Results represent the mean of three experiments ± S.D.

**Table 2. Effects of various C:N on exopolymer production**

| Sucrose | Peptone | Carbon to nitrogen ratio | Exopolymer (mg $L^{-1}$) |
|---|---|---|---|
| 2.5 % | 0.5 % | 14.68:1.00 | 115.16 ± 0.28 |
| | 0.6 % | 12.23:1.00 | 101.83 ± 0.76 |
| | 0.7 % | 10.48:1.00 | 87.16 ± 0.28 |
| | 0.8 % | 9.17:1.00 | 74.26 ± 0.25 |
| | 0.9 % | 8.15:1.00 | 68.26 ± 0.25 |
| | 1.0 % | 7.34:1.00 | 60.16 ± 0.15 |

Results represent the means of three experiments ± S.D

**Table 3. Experimental design generated with Design Expert 9.0 : the predicted and actual values of exopolymer production by *Bacillus clausii***

| Run | A:Seasalt (%) | B:pH | C:Temp °C | Predicted production value of Exopolymer (mg $L^{-1}$) | Actual value of Produced exopolymer (mg $L^{-1}$) |
|---|---|---|---|---|---|
| 1 | 5 | 7 | 37 | 410.00 | 410 |
| 2 | 5 | 7.5 | 45 | 364.92 | 376 |
| 3 | 2.5 | 7 | 45 | 291.08 | 281 |
| 4 | 5 | 7 | 37 | 410.00 | 410 |
| 5 | 5 | 7 | 37 | 410.00 | 410 |
| 6 | 7.5 | 6.5 | 37 | 305.17 | 306 |
| 7 | 7.5 | 7 | 25 | 319.49 | 328 |
| 8 | 5 | 7 | 37 | 410.00 | 410 |
| 9 | 5 | 6.5 | 25 | 314.18 | 305 |
| 10 | 2.5 | 7 | 25 | 273.76 | 276 |
| 11 | 7.5 | 7 | 45 | 348.67 | 348 |
| 12 | 5 | 6.5 | 45 | 342.33 | 342 |
| 13 | 5 | 7 | 37 | 410.00 | 410 |
| 14 | 2.5 | 7.5 | 37 | 278.83 | 278 |
| 15 | 5 | 7.5 | 25 | 346.57 | 345 |
| 16 | 7.5 | 7.5 | 37 | 326.68 | 318 |
| 17 | 2.5 | 6.5 | 37 | 247.32 | 256 |

**Table 4. ANOVA for response surface quadratic model**

| Source | Sum of Squares | Df | Mean Square | F Value | p-value Prob > F |
|---|---|---|---|---|---|
| **Model** | 46784.42 | 9 | 5198.27 | 67.25 | < 0.0001 |
| **A-Seasalt** | 5234.23 | 1 | 5234.23 | 67.71 | < 0.0001 |
| **B-pH** | 1482.36 | 1 | 1482.36 | 19.18 | 0.0032 |
| **C-Tem** | 1081.13 | 1 | 1081.13 | 13.99 | 0.0073 |
| **AB** | 25.00 | 1 | 25.00 | 0.32 | 0.5873 |
| **AC** | 35.88 | 1 | 35.88 | 0.46 | 0.5176 |
| **BC** | 24.51 | 1 | 24.51 | 0.32 | 0.5909 |
| **A2** | 25045.33 | 1 | 25045.33 | 324.00 | < 0.0001 |
| **B2** | 7921.64 | 1 | 7921.64 | 102.48 | < 0.0001 |
| **C2** | 2050.68 | 1 | 2050.68 | 26.53 | 0.0013 |
| **Lack of Fit** | 541.11 | 3 | 180.37 | | |

| | | | |
|---|---|---|---|
| **Std. Dev.** | 8.79 | **R-Squared** | 0.9886 |
| **Mean** | 341.71 | **Adj R-Squared** | 0.9739 |
| **C.V. %** | 2.57 | **Pred R-Squared** | 0.8104 |
| **PRESS** | 8972.12 | **Adeq Precision** | 24.124 |

**Table 5. Antibacterial activity**

| *Name of the bacteria* | 5 µg/ml | 10 µg/ml | 15 µg/ml | 20 µg/ml |
|---|---|---|---|---|
| *E.coli* | 4.3 ± 0.5 | 4.6 ± 0.5 | 5.3 ± 0.5 | 7.6 ± 0.5 |
| *S.dysenteriae* | 5.6 ± 0.5 | 6.3 ± 0.5 | 7.3 ± 0.5 | 8.3 ± 0.5 |
| *S.aureus* | 10.6 ± 0.5 | 12.6 ± 0.5 | 13.3 ± 0.5 | 14.3 ± 0.5 |
| *S.pyogens* | 6.3 ± 0.5 | 7.6 ± 0.5 | 8.3 ± 0.5 | 9.6 ± 0.5 |

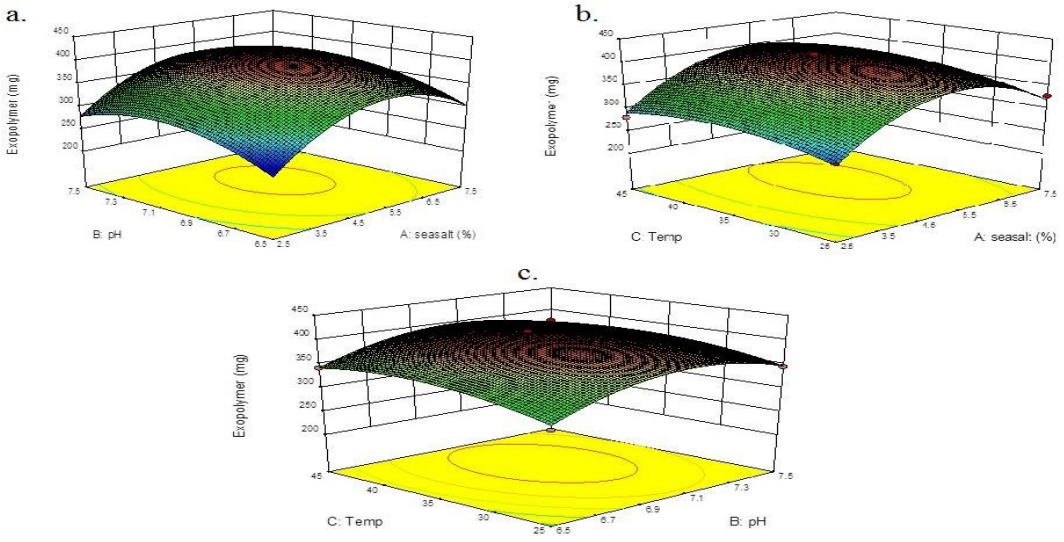
Results represent the means of three experiments ± S.D

$$(Y) = 408.604 + 25.8284\ A + 13.7451B + 11.625\ C$$

$$+ -2.5\ AB + 2.96569\ AC + -2.45098\ BC$$

$$+ -77.125\ A^2 + -43.375\ B^2 + -23.2292\ C^2$$

The lower value of CV 2.57 indicated a greater reliability and the probability values are 0.0001 (sea salt), 0.0032 (pH), 0.0073 (temperature) significant in exopolymer production (Table 4). The Pred R-Squared values (0.8104) are a reasonable agreement with the Adj R-Squared value (0.9739). A ratio of 24.124 indicates an adequate signal. There is only a 0.01% chance that an F-value this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. The goodness of fit of the model was checked by determination coefficient ($R^2$). In this case, the value of the determination coefficient ($R^2$ = 0.99) indicates the significance of the model. The coefficient of variation (CV) indicates the degree of precision with which the experiments were compared. The interaction effect of variables on polysaccharide production was shown in Figure 1. *Shouchella clausii* produces 410 mg $L^{-1}$ of exopolymer in the basal salt medium supplemented with 5 % sea salt (pH 7), at 35 °C.




The maximum production of exopolymer (410 mg L−1) for *Shouchella clausii* was observed in a medium supplied with sucrose (2.5 %) and peptone (0.5 %), sea salt (7.5 %), pH 7.0 and incubation temperature 37 °C

**Figure 1. Interactive effects of variables on the exopolymer production. (A) pH and salt (B) Temperature and salt (C) pH and Temperature**

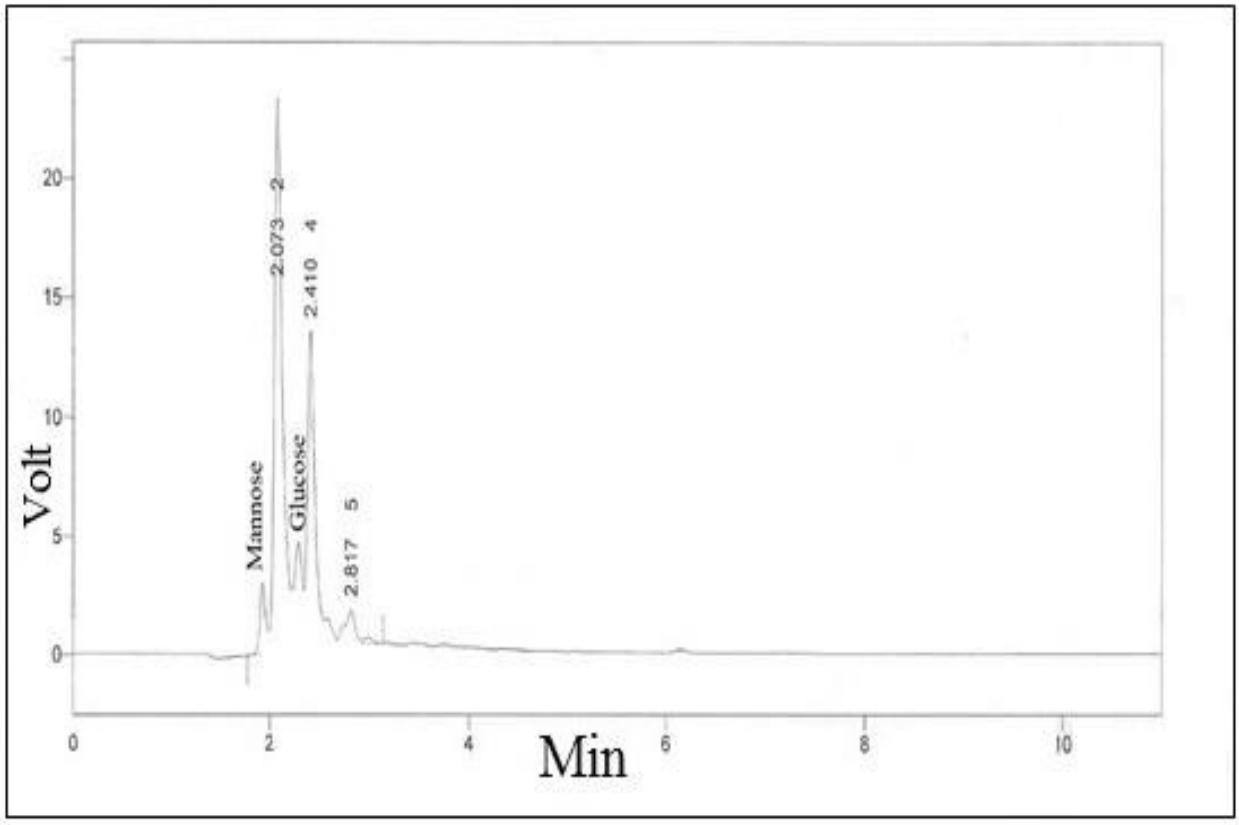



**Figure 2 HPLC analysis of exopolymer produced by *Shouchella clausii*** The HPLC analysis of exopolymer produced by *Shouchella clausii*. Contains glucose and mannose as major sugars

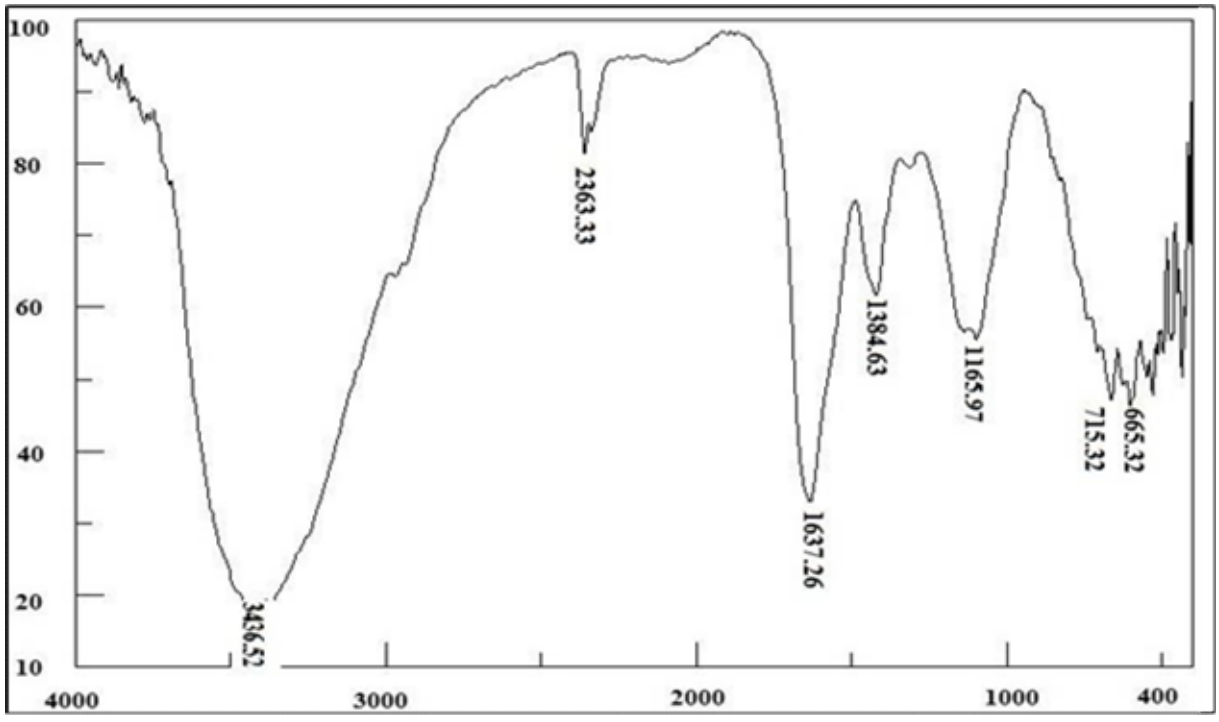



**Figure 3 FTIR analysis of exopolymer produced by *Shouchella clausii*** The stretchings at 800.40 $cm^{-1}$ indicates for sulphate group, 1112.85 $cm^{-1}$ - 1191.93 $cm^{-1}$ for sugars 3315.41 $cm^{-1}$ and 3203.54 $cm^{-1}$ indicates for $NH_2$ group.

**Characterization of bacterial polysaccharide:** The polysaccharide contains sugars (77.85 %), proteins (2.5 %), uronic acids (4 %), and sulphates (12.7 %). HPLC analysis showed that exopolymer consists of glucose, mannose and rhamnose (Fig 2).

**FTIR analysis of exopolysaccharide:** The stretching at 715.32$cm^{-1}$ indicates the presence of sulphate group (Parikh and Madamwar 2006). The peaks at 1165.97 $cm^{-1}$ corresponded for characteristic sugar derivatives. The peaks at 1637.26 $cm^{-1}$ could be attributed to the COOH group and the medium stretching at 3436.52 $cm^{-1}$ indicates the presence of $NH_2$ group (Fig 3).

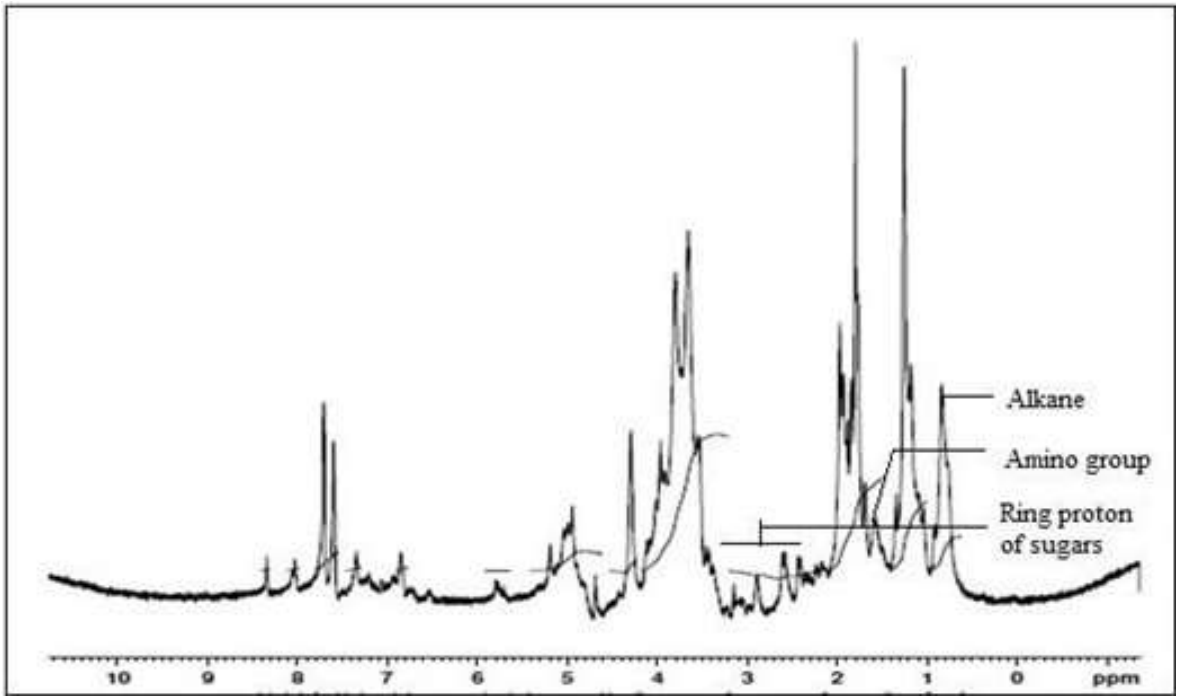



**Figure 4 NMR Proton analysis of exopolymer** The carbohydrate finger print region were observed at 3.397- 4.291-ppm and the stretching for sulphates were observed at 2.171 ppm. The signals for amino group of protein were observed at 1.301-1.375 ppm.

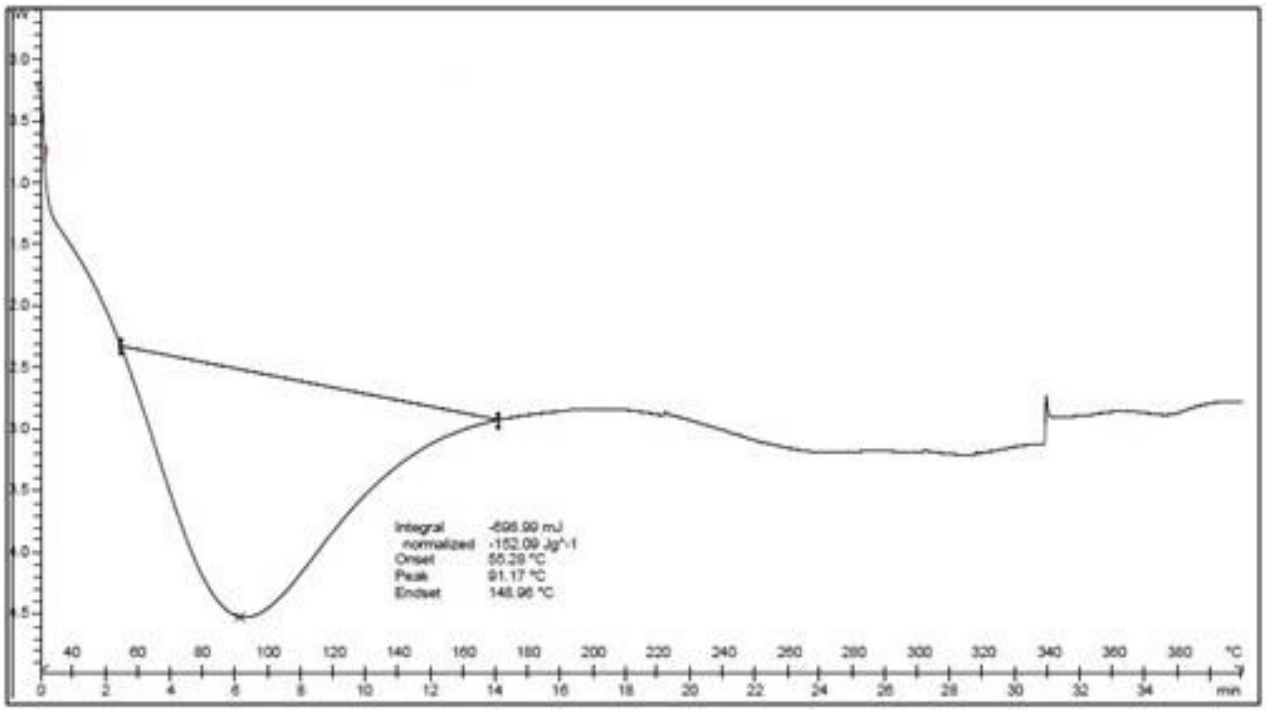



**Figure 5. DSC analysis of exopolymer. DSC analysis showed the melting point of the exopolymer was 260.45 °C.**

NMR analysis of exopolysaccharide. In comparison with carbohydrate research database (www.glyco.ac.ru), The signals obtained between 0.833 ppm corresponds for the alkane, and the signals for alkene was observed at 1.179- 1.342 ppm. The proton signals arising from the methyl protons of the 6-deoxy sugars were seen at 1.796-1.964 ppm. On the other hand, stretching of N-H group of protein was observed at 1.342 ppm. The ring proton region or finger print region of sugar moieties was observed at 3.449-4.687 ppm (Fig 4).

**DSC analysis of exopolysaccharide:** DSC analysis showed a significant exothermic thermal transition (Fig 5). The polysaccharide of *Shouchella clausii* exhibited a single narrower peak with a maximum melting temperature ($T_m$) of 91.17°C. The onset transition temperature was found at 55.28°C. About 22.79 % weight loss was observed during I phase of degradation for

the exopolymer of *Shouchella clausii* at 80°C - 160°C and the second phase of degradation of exopolymer (85.45 %) was observed with maximum loss at ≥ 340°C. UV-Visible absorption spectrum of nano particles showed an absorption maximum at 31 nm as shown in Figure 6. The size and shape of Copper nano particles recorded in the scanning electron microscope is represented in Figure 7. The average size of the particle was 35 nm.

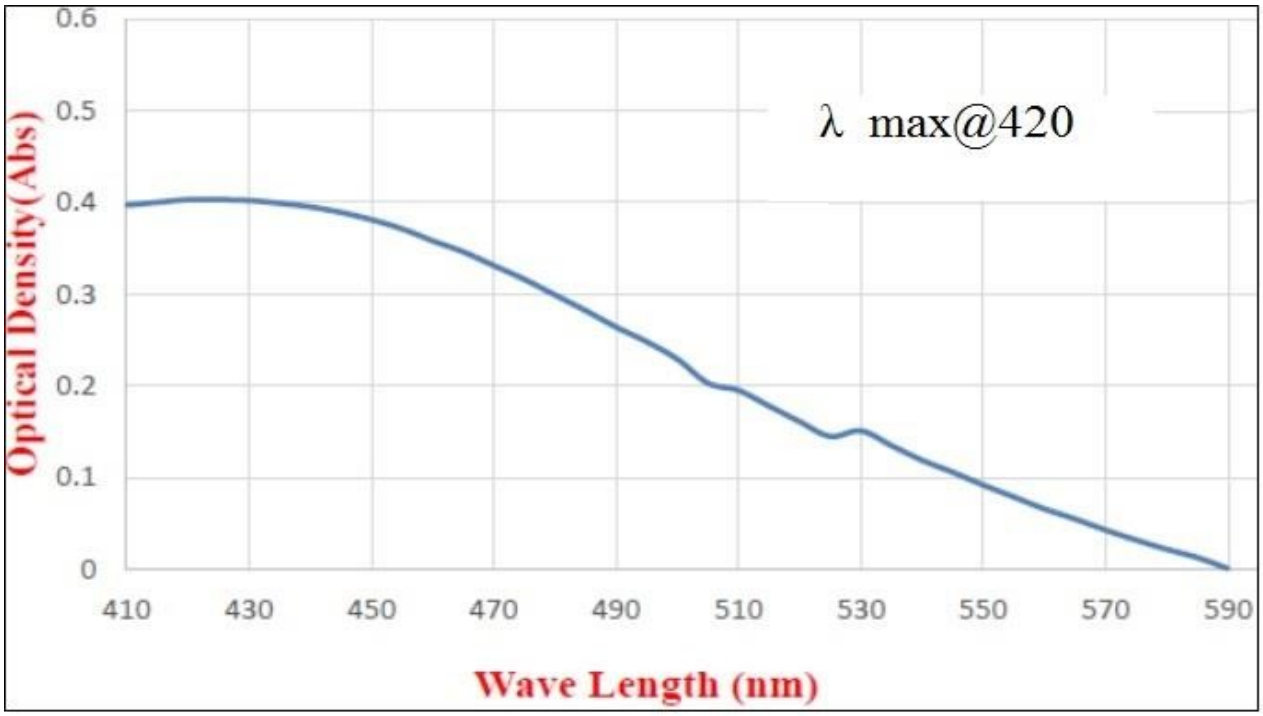



**Figure 6 UV-Visible absorption spectrum of Copper nanoparticles Figure 7 SEM analyses of Copper nanoparticles**

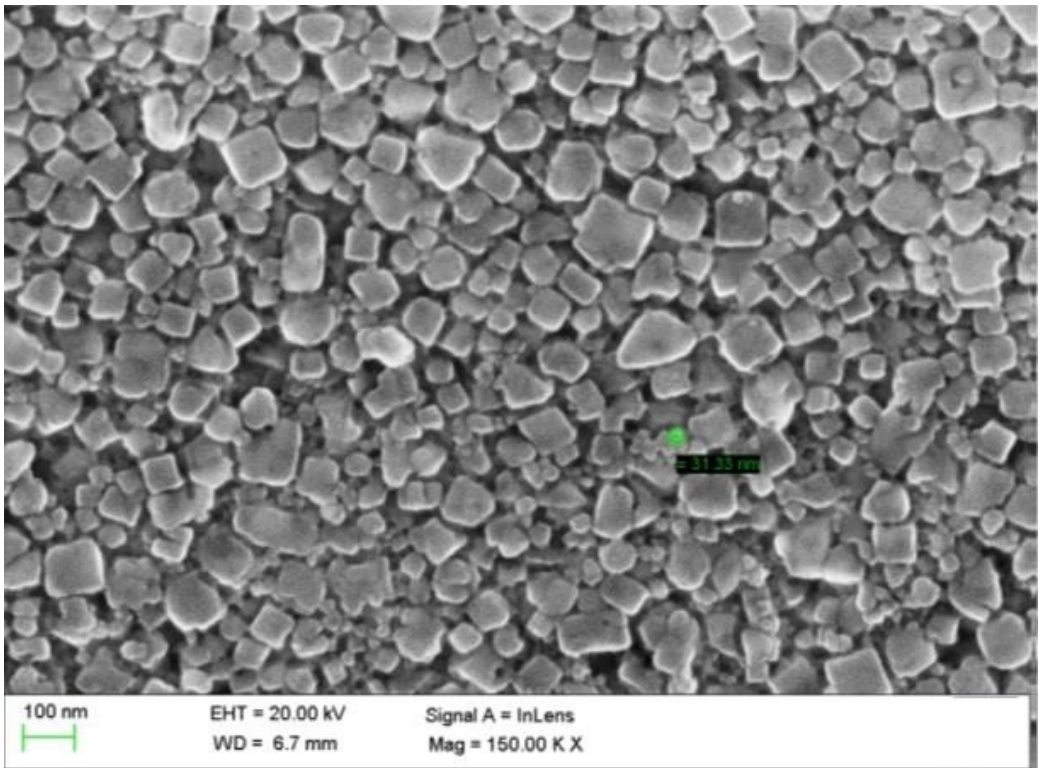



**Figure 7**

**Applications of polysaccharide:** The results of antibacterial activity are depicted in Table 5, which showed that the antibacterial activity differed based on the test strains. A considerable level of antibacterial activity was noticed. The polysaccharide based NP's at the concentration of 20 µg/ml shown highest level of antibacterial activity and the level of zone of inhibition increases gradually when the concentration increasing from 5 to 20 µg/ml.

**DPPH radical scavenging assay:** The scavenging activity of the standard L-Ascorbic acid was found 37.32 % at 0.1 mg/mL and 67.05 % at 1 mg/mL, whereas exopolymer - CuNPS showed antioxidant activity of 21.72 % at 0.1 mg/mL and 35.95 % at 1 mg/mL (Figure 8).

**Reducing power assay:** Figure 9 shows the reducing power of polysaccharide based NPs as a function of its concentration. L-Ascorbic acid as positive control in this test, had absorbance value of at 0.89 at 1.0 mg/mL. At low concentration (0.2 mg/mL), the absorbance is found low (L-Ascorbic acid (0.156), exopolymer AgNPs (0.07).The reducing power of the exopolymer CuNPs is increased with their concentration. Whereas, higher absorbance (A700 0.495) is found at higher concentration at 1 mg/mL.

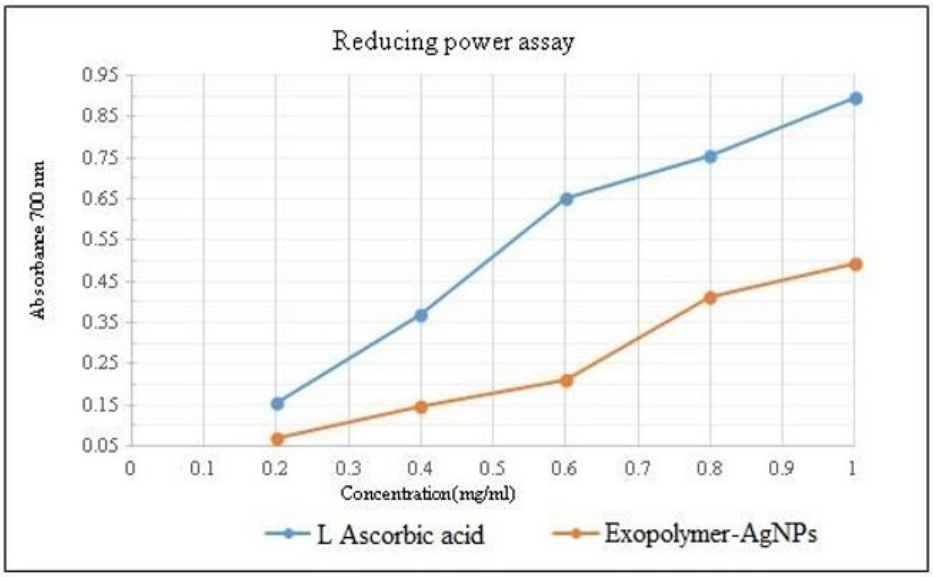



**Figure 8 DPPH radical scavenging activity of exopolymer based AgNPs. DPPH radical scavenging activity increases depend on the concentration of exopolymer**

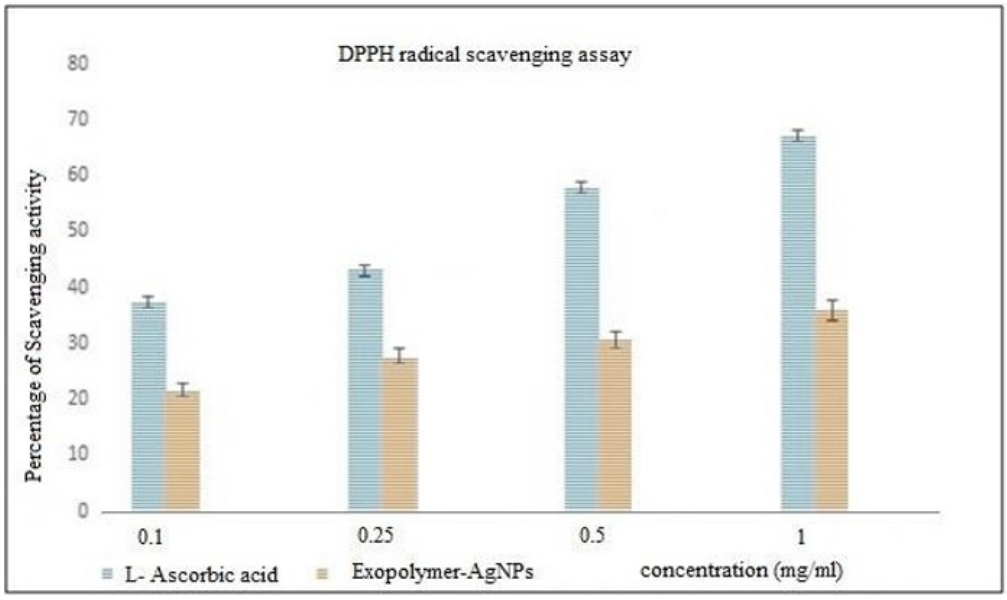



**Figure 9 Reducing power activity of exopolymer based AgNPs. Reducing power activity increases depend on the concentration of exopolymer**

# DISCUSSION

The presence of sticky texture is a key factor for the identification of polysaccharide producing bacteria and it was identified as *Shouchella clausii* by 16S rRNA gene sequencing method. It has been clearly shown that the carbon source and the concentration have an impact on exopolymer production (Cerning J 1999). *B.lichniformis* SVD1 (Dyk *et al*., 2012) and *Bacillus thermoantarcticus* (Manca *et al*., 1996) produced 54.96 $mgL^{-1}$, 100 $mgL^{-1}$ of exopolymer respectively upon using 4 % sucrose in basal salt medium. However, *Bacillus cereus* GU812900 produced 426.06 mg $L^{-1}$ of exopolymer when 1 % sucrose was added in basal salt medium (Bragadeeswaran *et al*., 1996). In the present study, *Shouchella clausii* produce large amount of exopolymer when providing peptone as a source of nitrogen. In general, the bacteria produced high amount of exopolymer while using organic nitrogen sources (peptone and yeast extract) in basal salt medium (Result not given) than inorganic sources (ammonium chloride and ammonium nitrate). In case of inorganic nitrogen source that may decrease the growth and production by affecting the

enzymes (Gandhi *et al*., 1997). Findings in the present study are consistent with the findings of Gorret *et al*., 2001 showing that exopolymer production was high at a low nitrogen concentration. Carbon to nitrogen ratio in the growth medium plays an important role in exopolymer production (Scheepe-Leberkuhne and Wagner 1986).There are similarities in the distribution of sugar in exopolymer between the present study and those described by Kennedy and Sutherland 1987.The sugars like glucose, mannose, galactose, xylose, and ribose were predominantly found in the exopolymer of marine bacteria. The low protein and uronic acid content was similar to *Bacillus licheniformis* (0.6 %), *Bacillus cereus* (1.85 %) (Antonio *et al*., 2013, Bragadeeswaran *et al*., 1996) and *Bacillus licheniformis* (1.3 %) (Antonio *et al*., 2013) respectively. The high sulphates in exopolymer were especially interesting. This finding, while preliminary, suggests that it was an unusual component of bacterial polysaccharide contributing polyanionic nature to exopolymer (Decho 1990). The consensus opinion was the sulphates were probably present only in cyanobacteria and archaea (Philipps and Vincenzini M 1998). Nevertheless, the exopolymer contains high amount of sulphates (15 %). It was in consistent with marine bacterium like *Bacillus marinus* (20.2 %) and *Halomonas eurihalina* V2-7 (20.7 %) (Osama *et al*., 2015, Calvo *et al*., 2002). Comparing with the so far published s FTIR spectrum results of the exopolymer a characteristic carbohydrate finger print region (1200 to 1400 $cm^{-1}$) is consistent with *B.licheniformis* and *B.amyloliquefaciens* (Antonio *et al*., 2013 , Prasad Rao *et al*., 2013). The stretching at 3011.30 $cm^{-1}$ represents the CH3-CH2 group of sugar.Phase I degradation in DSC analysis was due to the evaporation of water during heating process while second phase of degradation was attributable to thermal decomposition same as other study (Parikh and Madamwar, 2006). The biogenic copper nanoparticles in the well diffused to surrounding and showed excellent antibacterial activities by prevented the growth of the bacterial cell. Findings of antimicrobial activity of copper nano particles in the present study are consistent with the findings of Ashkarran *et al*., 2012; Martínez-Robles *et al*., 2016. Bactericidal property of the polymer copper nanoparticles was predicted that there is a strong interaction between the thiol group of membrane proteins and copper ions. As a result the copper ions enter into cells and affects the DNA replication followed by the denature of membrane proteins (Qayyum *et al*., 2017).

In an earlier report, Osama *et al*., (2015) found the polysaccharide of *Bacillus marinus* exhibits 79.00 % scavenging activity at 1mg/mL. The DPPH scavenging activity of extract of the marine *Bacillus* sp. JS showed 86.51 % at 2 mg/mL (Abdel Wahab *et al*., 2013). The antioxidant activity of native polysaccharide of bacteria and CuNPs scavenged DPPH radicals (DineshBabu Manikandan *et al*., 2021). This was probably due to the presence of proteins, amino acids, and other elements in native exopolymer (Liu *et al*., 2010). The reducing power of a compound may serve as a significant indicator of its antioxidant activity (Kumar *et al*., 2004). However, the carbohydrate fraction of exopolysaccharide produced by *Bacillus amyloliquefaciens* C-1 was composed of D-glucose, D-mannose, D-galactose, and D- arabinose which shows reducing activity (0.82) at 5 mg/mL (Yang *et al*., 2015).The absorbance of reducing power assay for the exopolysaccharide produced by *Bacillus licheniformis* UD061 was observed 0.34 at 250 mg/mL (Fang *et al*., 2013). Mahendran *et al*., (2013) reported that an increase in concentration associated with high absorbance indicated high reducing power. The reducing power might be attributed to the functional groups in the exopolymer, which can donate electrons to reduce the radicals to a more stable form or react with the free radicals to terminate the radical chain reaction (Leung *et al*., 2009).

# CONCLUSION

*Shouchella clausii* MZ128362 was successfully used for the production of polysaccharide. The biogenically synthesized nanoparticles using bacterial polysaccharide was confirmed by spectrophotometrically and microscopically. The SEM analysis shows that spherical CuNPs with mean diameter of 31 nm capped by a thin layer of EPS. The prepared CuNPs were displays obvious antibacterial, and anti oxidant property.

**Abbreviations**

$CuSO_4.5H_2O$- Copper Nitrate
CuNPs- Copper Nanoparticles
DSC- Differential scanning calorimeter DPPH- 2,2-diphenyl-1-picrylhydrazyl.
FTIR- Fourier-transform infrared spectroscopy
NMR- Nuclear magnetic resonance
NPs- Nanoparticles

ROS- Reactive oxygen species

Declarations

**Ethics approval and consent to participate:** Not Applicable

Research involving Human Participants and/or Animals: **Not Applicable**

**Informed Consent for publication: Not applicable**

Availability of the Data and material

All data generated or analyzed during this study are included in this published article

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**Authors Contribution:** KP carried out the isolation of bacteria; synthesis and characterization of the exopolymer.PS carried out the computational experiments and drafted the manuscript. All authors read and approved the final manuscript.

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