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Biosynthesis Of Copper Oxide Nanoparticles From Martynia Annua Aqueous Leaf Extract, Its Antioxidant Potential And Anti-Bacterial Ability Against Selected Wound Pathogens

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ABSTRACT

The present study report an efficient method for synthesis of copper oxide nanoparticles using aqueous leaf extract of Martynia annua. The induction of copper ion reduction process was affirmed based on the colour change of reaction mixture to deep black and the synthesis of nanoparticles was further confirmed using UV visible spectroscopy in the wavelength range of 234 nm. FTIR spectroscopy of synthesized CuO nanopoarticles reveals that the phytochemicals present in the aqueous leaf extract capped, stabilized and functionalized the nanoparticles during synthesis. The XRD analysis disseminates the crystalline nature of CuO which is propionate with increased sharpness of distinctive peaks observed during the formation of copper oxide nanoparticle. SEM image of synthesized CuO nanoparticles explain that the nanoparticles are small, well crystallinised with smooth texture of cubes and rods in shape with some degree of aggregation. The presence of moderate signal for copper ions at 3 Kev was observed during EDX analysis, which represent that copper ions are reduced during the formation of copper oxide nanoparticles. The average particle size of synthesized copper oxide nanoparticle is 112.3 nm. The PDI value of biosynthesized CuO nanoparticle is -36.2, which is associated with low particle size and least value of poly dispersity index, indicates good stability of nanoparticles. The biosynthesized nanoparticles possess well regulated DPPH scavenging activity and reducing power assay when compared to standard. The antioxidant potential of synthesized nanoparticles is entirely based on dose dependent activity. The antibacterial activity of synthesized copper oxide nanoparticle is impressive against tested wound pathogen when compared to standard. It concluded that, the synthesized nanoparticle can be incorporated as a constituent in commercial wound healing gel by following appropriate clinical trial.

KEYWORDS: Synthesis and characterization of copper oxide nanoparticles.

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INTRODUCTION

Nanotechnology is a great boon for humanity because its importance has overspread the way for numerous applications which includes therapeutics, catalysis, microelectronics and biological sensors. It also finds major impact on areas viz., optical, mechanical, electrical and health care due to their small size and large surface area to volume ratio. Nanoparticles are considered as a scientific revolution in the present century, which are required for the technological revolution in modern day science and engineering. It develops rapidly in several subjects like chemistry, physics, engineering and medicine (Berra et al., 2018).

Nanotechnology is concern with the synthesis of different sizes, shape, divergance and chemical composition of metal and metal oxide nanoparticles. Nanoparticles are the major building blocks of nanotechnology, which are the clusters of atoms in the sizerange between 1–100 nm. Nanosized objects and their elaboration play a major role in development of science and technology, modern research, pharmacy, food, biomedical sciences, pharmaceuticals, chemistry, chemical industry, energy sciences, cosmetics, environmental health and space industry (Iravani et al., 2011).

Metal oxide nanoparticles had increased the efficiency of different industries while it finds various applications with multiple functions, as well as low cost with high life time efficiency. Synthesis of metal oxide nanoparticles using green chemistry approach involves the usage of corresponding metal oxide ion salt through reduction processes there by reducing the risk of hazardous substances which threaten the human health and environment (Saif et al., 2016).

Copper oxide nanoparticles find enormous applications in various modern technologies like solar cell, optical and catalysis. It is a promising material for semiconductor and photovoltaic cells due to their low band. It has also been used as an organic dye degradation material in different industries (Khatami et al., 2017).

Copper oxide nanoparticles are most commonly used catalysts in different cross coupling reactions. It has a major role in oil industry to remove the undesired products from oil. Specifically, copper oxide is a good conductor of heat or electricity and it is cheaper than silver and copper. Further, it has extended the application as water purifier, antimicrobial agent and high temperature super conductors in batteries and gas sensors. Copper oxide nanoparticles have a high surface area to volume ratio that makes them interact easily with other materials (Sivaraj et al., 2014).

Having a moderate band gap, good catalytic activity and high optical transparency which further extends their industrial applications. In this context, the present study was designed to synthesis of copper oxide nanoparticles using aqueous leaf extract of Martynia annua.

MATERIALS AND METHODS

Synthesis and characterization of copper oxide nanoparticles

In the process of biosynthesis of copper oxide nanoparticles 5mL of freshly prepared plant extract was mixed with 5mL of CuSO4(1 mM) solution in a conical flask and stirred well for a day in room temperature. The colour change of the fused mixture was observed and recorded. Preliminary confirmation of synthesized copper oxide nanoparticle was done by using UV-Vis spectrometer (Shimadzu- 1800) operating in the wavelength range between 200-800 nm.

The biosynthesized copper oxide nanoparticles was further characterized using different analytical techniques. The functional group present in the surface of synthesized copper oxide was confirmed using FTIR spectroscopy.

The average particle size, surface morphology and elemental composition of synthesized nanoparticles was studied using Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) spectroscopy (SIGMAmodel,CASLZEISS,German) operating at an accelerating voltage of 3.00 KV. The particle size distribution of synthesized nanoparticles was evaluated using dynamic light scattering measurements (DLS) and zeta potential analysis was conducted using Malvern zetasizer neroseries compact scattering spectrometer (Malvern Instruments Ltd., Malvern, UK) for exploring surface charge of synthesized nanoparticles.

Determination of antioxidant activity of synthesized copper oxide nanoparticles

DPPH free radical scavenging activity

The free radical scavenging ability of the synthesized copper oxide was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH (Nagdu Dhruti, 2009). Different aliquots of copper oxide nanoparticle in suspension (10-200µL/mL) were thoroughly mixed with 0.1 mM DPPH in 1 mL of methanol solution. The reaction mixture was allowed to stand for 30 min in dark and the absorbance was measured at 517 nm. An equal amount of methanol with DPPH was used as and blank and the ascorbic acid was used as standard. Percentage inhibition of DPPH free radical was calculated based on the blank reading, which contains DPPH and methanol without any extract using the following equation:

$$\text{DPPH Scavenging activity (\%)} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

Where Ablank was the absorbance of the blank and Asample was the absorbance of the sample in the presence of the CuO NP. The antioxidant activity of the CuO NP was expressed as IC50. The IC50value was defined as the concentration (in µg/mL) of CuO NP that inhibits the formation of fifty percent DPPH free radicals.

Ferric reducing antioxidant power

The FRAP assay is another method for detecting antioxidant capacity. FRAP reagent was prepared using 300mM acetate buffer (pH3.6), 10mMTPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM hydrochloric acid and 20 mM iron (III) chloride. All the prepared solution was mixed in ratio of 10:1:1. The freshly prepared FRAP reagent (150 µL) was mixed with 20 µL of sample, 20 µL of ascorbic acid(positive control) and 20 µL of distilled water (blank).

Reducing antioxidant assay was performed by adding3.995mL of working FRAP reagent with different concentrations (10-200 µg/mL) of the appropriately diluted CuO NP and mixed thoroughly. An intense blue color complex was formed when ferric tripyridyl triazine (Fe^{3+} TPTZ) complex was reduced to ferrous form (Fe^{2+}) and the absorbance was recorded at 593nm against reagent blank (3.995mL FRAP reagent + 5 µL distilled water) after 30 min of incubation at 37ºC. All the determinations were performed in triplicates and the values are expressed as ascorbic acid equivalents in µg per mg of CuO NP (Lakshmanan et al., 2018).

Antibacterial activity

Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of synthesized CuO NPs was determined by following the standard protocol (Arasu et al., 2013). The synthesized CuO NPs was mixed with double distilled water and sterilized using 0.2µ filter discs. The sterilized solution was serially diluted to two fold of known concentrations (6.25, 12.5, 25, 50, 100 µg/mL) using Muller-Hinton broth with appropriate control in96- well micro titer plates. Further, 5µl of the newly cultured bacterial suspensions of the test organisms (Eschericiacoli, Staphylococcus aureus, Streptococcussp, Bacillus sp and Enterococcus faecalis) (1.5 × 106CFU/mL) was transferred to 200 µL of sterilized MHB. Amoxicillin was used as positive control.

Follows the proper mixing, the 96-well micro titer plates was covered with a sterile plate sealer and incubated at 37ºC for 20 hr. MIC is defined as the lowest concentration of the nanoparticles that inhibit the growth of the test cultures. The experiment was repeated thrice to confirm the antibacterial ability of synthesized copper oxide nanoparticles.

Statistical analysis

The datas of all experimental parameter was analyzed using Minitab 16 statistical software which utilizes Statistical One Way Analysis of Variance (ANOVA) followed by Dunnett's test. The experimental groups would be compared with respective control groups and the values are expressed as mean±Standard Error Mean (S.E.M).*Pvalue< 0.05 was considered as significant.

RESULTS AND DISCUSSION

Synthesis, preliminary confirmation and characterization

The preliminary confirmation of synthesized copper oxide nanoparticles was made based on the colour change of reaction mixture into deep black once the reaction was finished (Fig.1a). The synthesized nanoparticles was filtered and centrifuged at 10,000 rpm for about 30 minutes. Leaf extract act as reducing and stabilizing agent, which is responsible for the development of the nanoparticles and also minimize the aggregation of nanoparticles by capping them with aldehyde and ketone groups (Santhoshkumar et al., 2017). The formation and stability of synthesized copper oxide nanoparticles was further confirmed by using UV-vis spectrophotometer. The curve in the UV spectrum at 234nm relates with surface Plasmon vibration of copper oxide nanoparticles and the broadness of the absorption peak are due to the wide size distribution of these nanoparticles (Fig. 1b) (Saif et al., 2016). Similar results were reported in biosynthesis of copper oxide nanoparticles using the leaf extract of Drypetes sepiaria (Palajonna Narasaiah et al., 2017).

FTIR spectrum of $CuSO_4$ shows the absorption peaks at 3647.14cm-1 were shifted to 3500.56 – 3213.19 cm-1, 3361.69 cm-1– 3278.76 cm-1is assigned to OH stretch which ensures the presence of hydroxyl group. The peak value at 1768.60 -1670.24 cm-1 is associated with C=O indicates the presence of aldehydes and unsaturated esters. The other peak in the range of 1681.81-1649.09 is related to C=C that confirm the presence of aromatic carbon. The stretch in the peak range between 1338.51-1315.36cm-1 represent C-N linkage that ensures the presence of aromatic amenes (Fig. 1c).

FTIR spectrum of copper oxide nanoparticles registered their absorption peaks at 3512.13-3284.55 cm-1 which is shifted to 3317.34-2686.66 cm-1, 2686.66 cm-1, 1350.08-1309.58cm-1and1326.93-1261.36 cm-1represent hydroxyl (OH) group. The peak in the ranges of 3386.77-3284.55cm-1 shifted to 2937.38-2885.31cm-1,1662.52-1585.38cm-1and 1585.38 cm-1 is related with N-H bond of primary amenes. Whereas, the peak values of 2686.66cm-1shifted to3317.34-3284.55cm-1and1662.52-1585.38cm-1reveals C-H and N-H bonds of alkyl halides and primary amines respectively (Fig. 1d).

The result of FTIR spectrum reveals that the phytochemicals present in the leaf extract is capped, stabilized and functionalized the nanoparticles during synthesis. This finding identify that the biosynthesized CuONPs can be used for different applications after prolonged storage. The results are correlates with the previous report on synthesis, characterization of copper oxide nanopowders and their use in nanofluids for enhancement of thermal conductivity (Prakash and Diwan, 2015).

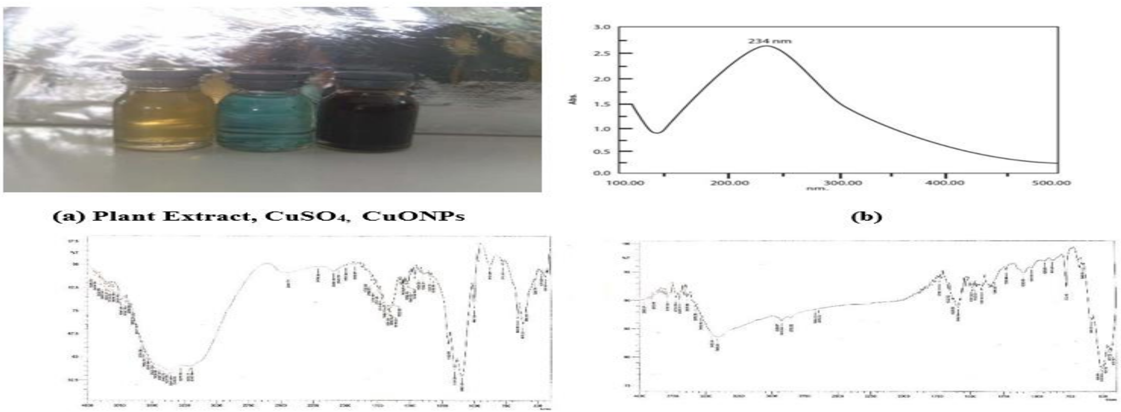


Fig 1. Synthesis, confirmation and characterisation of copper oxide nnanoparticles using aqueous leaf extract of Martynia annua (a) Synthesis of copper oxide nanoparticles, (b) UV-visible spectrum of biosynthesized copper oxidenanoparticles, (c) FTIR Spectrum of biosynthesized copper oxide nanoparticles (d) FTIR Spectrum of

biosynthesized copper oxide nanoparticles from Martynia annua

A powder X-ray diffraction analysis entrust an insight about the crystalinity nature of the nanoparticles, it is the major factor which influence on solubility and stability behavior of the nanoparticle in colloidal suspension (Yelil Arasi et al., 2012). Differences in the peak size may due to different route of synthesis, imperfect crystallization and nature of strain. The XRD analysis of biosynthesized copper oxide nanoparticles formulation exhibit diffraction peaks in 2θ angle of 38.32°, 35.32°and 21.95° (Fig.2a). It reveals that the increase in the degree of crystalline nature is propionate with increase in the sharpness of the distinctive peaks observed during formation of copper oxide nanoparticle using Martynia annua fresh leaf extract. This result is in accordance with the results of green synthesis and characterization of copper nanoparticles using Azadirachta indica leaves (Nagar and Devra, 2018).

The surface morphology of green synthesized copper oxide nanoparticles was analyzed using Scanning Electron Microscopy. The SEM image of the synthesized nanoparticles reveals that they are small in size, uniformly well crystallized with smooth texture of cubes and rods in shape with some degree of aggregation (Fig. 2b). The shape and morphology of the nanoparticles are dependent on reducing agent as well as stabilizing agent. This data are in full agreement with recent data of studies on green synthesis of CuO nanoparticles using Euphorbia maculate extract as photocatalyst for the degradation of organic pollutants under UV irradiation (Pakzad, 2019).

Energy dispersive X- ray spectroscopy (EDX) is a quantitative technique used for elemental analysis of chemicals composition on the surface of the synthesized nanoparticles. EDX analysis reveals the presence of moderate elemental signal for copper oxide at 3 KeV, which is typical for the absorption of metallic copper nano crystallites due to surface plasma resonance. Further,EDX analysis substantiates the presence of

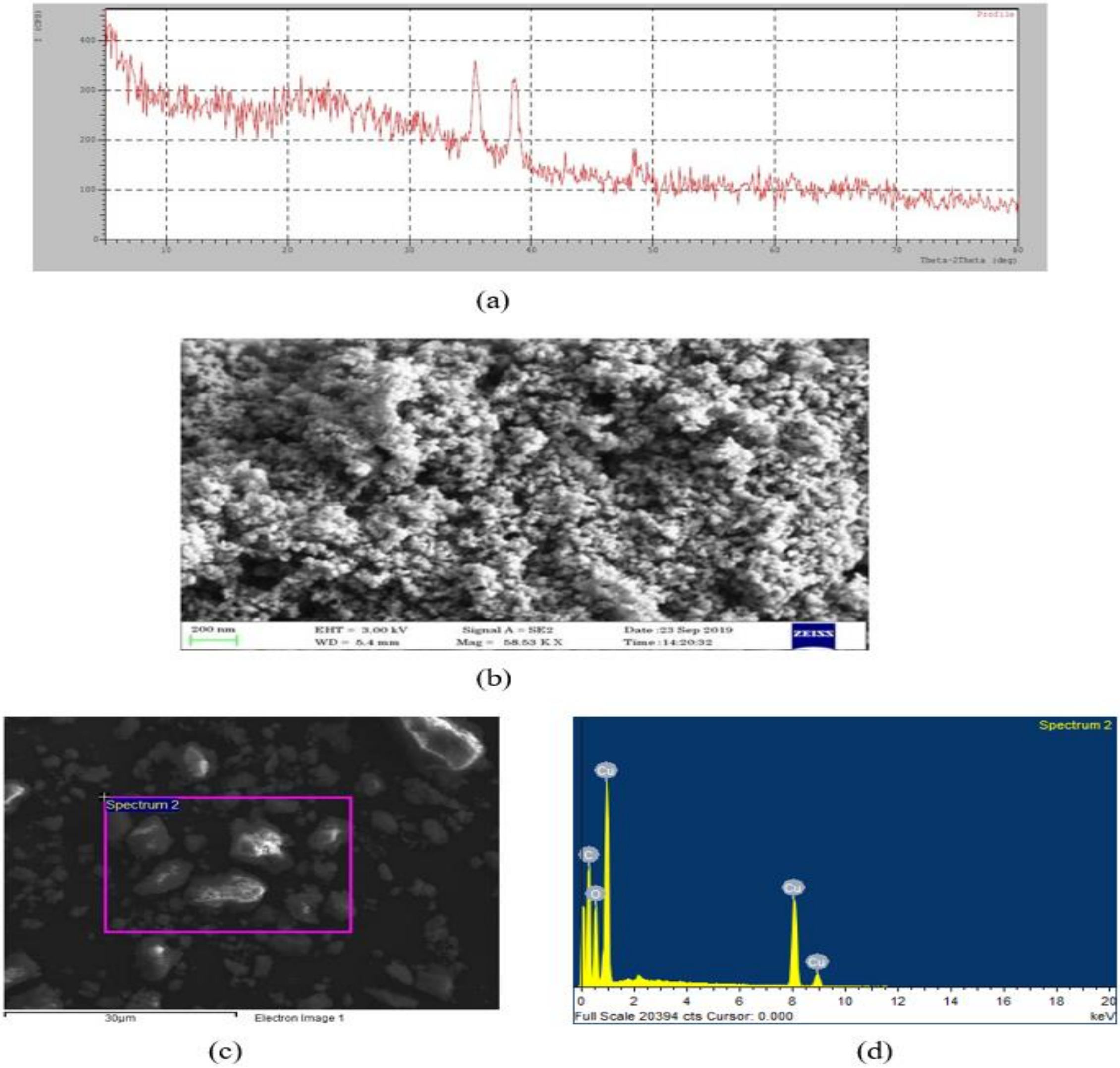


Fig. 2. Analytical characterization of copper oxide nanoparticles synthesized using aqueous leaf extract of Martynia annua (a) X-ray diffraction pattern of biosynthesized copper oxide nanoparticles, (b) Scanning electron microscopic image of biosynthesized copper oxide nanoparticles, (c) EDAX image of copper oxide nanoparticles with bioorganic components (d) EDAX pattern of biosynthesized copper oxide nanoparticles

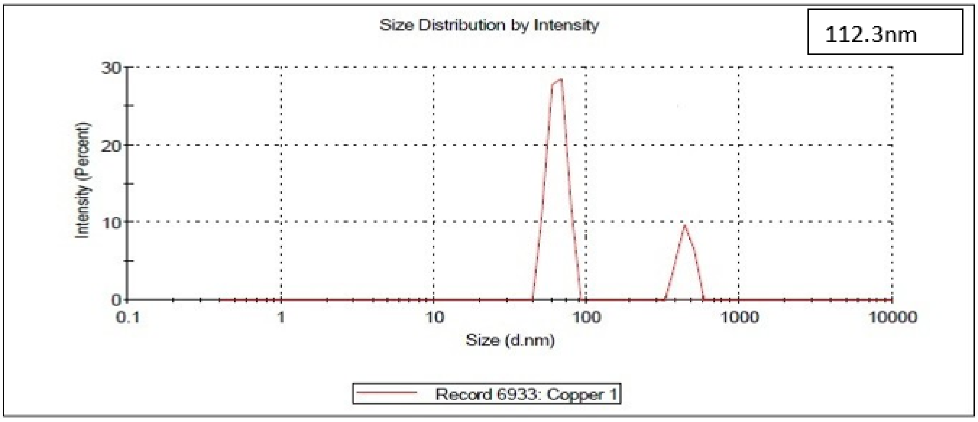
copper and oxygen as a predominant constituent of biosynthesized samples. The biosynthesized sample contains 34.41% copper, 22.23% oxygen and 43.36% carbon (Fig.2c,d).This results correlates with the results of phytomediated synthesis of copper oxide nanoparticles using Camelliasinensis and Prunusafricana bark.

Dynamic light scattering (DLS) analysis is used to determine the average particle size distribution profile of synthesized nanoparticles and capping agent enveloped the metallic particles along with the particular size of metallic core. The particle size distribution has most important characteristic of nanoparticles which affect the in vitro fate of nanoparticle. The particle size was measured using Malvern particle size analyzer. The results of the study reveal that the average size distribution of CuO nanoparticles is 112.3 nm(Fig. 3a).

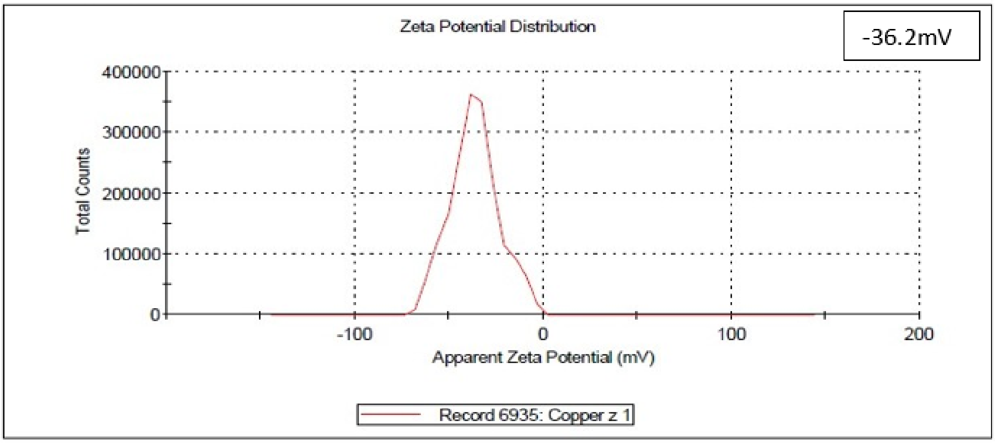
The zeta potential is an important parameter and provides information about the stability and state of the nanoparticle in liquid suspension. Nanoparticles with high zeta potential posesess increased stability due to larger electrostatic repulsion between particles (Selvarararaja et al.,2017).The zeta potential value of biosynthesized CuO nanoparticle is -36.2mV, which is associated with low particle size and least valueof poly dispersity index, it provide good stability of nanoparticles (Fig. 3b). This result isin accordance with the results of green synthesized cupric oxide nanoparticles using the water extract of Murrya koenigi and its photocatalytic activity (Anandhavalli, 2015).

DPPH free radical scavenging activity

Free radicals are created by several metabolic pathways in humans. Due to the release of reactive oxygen species which interact with molecules of other unrelated metabolic pathways and these free radicals are unstable in nature, as leads to cellular damage (Ravindra et al. 2010).Therefore these free radicals are blame for variety of degenerative diseases and lowered immune function system of human. In order to combat free radicals, medicinal plants with phenolic contents are employed as antioxidants (Elsabahy and Wooley, 2013). Donating or accepting electrons from the abundant reactive oxygen species antioxidants scavenge oxygen derived free radicals and prevent numerous diseases (Liu et al., 2018). Further, the binding properties of transition metal ion catalysed by free radicals enhances the radical scavenging ability which is the thought



(a)



(b)

Fig. 3. Dynamic light scattering ananlysis (DLS) of biosynthesized copper oxide nanoparticles using aqueous leaf extract of Martynia annua (a) Particle size distribution of copper oxide nanoparticles (b) Zeta potential spectrum of

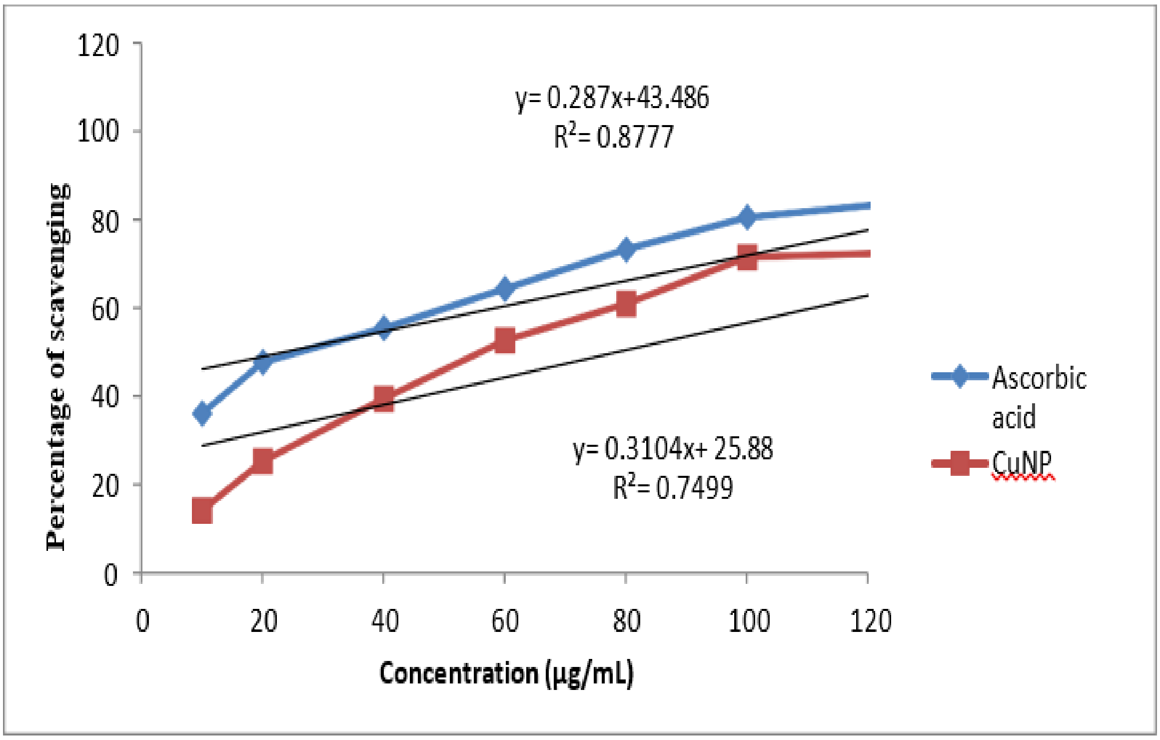
biosynthesized copper oxide nanoparticles.

providing mechanism behind the antioxidant activity of inorganic nanoparticles (Mittal et al., 2014).
DPPH is more stable, well known free radical based on its reduction of accepting hydrogen or electron of donors. The IC50 value of ascorbic acid and synthesized CuONPs was found to be 22.69µg/mLand77.70µg/mL respectively (Table1; Fig.4a). The biosynthesized copper oxide nanoparticles exhibit potent DPPH scavenging activity when compared with standard ascorbic acid. It was observed that a significant decrease in the concentration of DPPH free radical due to the scavenging ability of synthesized CuO NPs. The results revealed that the synthesized CuO NPs registered high antioxidant activity, it may due to capped phenolic compounds of the plant extract. Phenolic group facilitates the conversion of $CuSO4$ into CuONPs which is based on its electron donating ability (Alamelumangai et al., 2014). Das et al., (2020)) reported similar kind of findings that are explained the CuO nanoparticle synthesized from Camellia sinensis and Prunus africana contain polyphenols and other phytochemicals which leads significant antioxidant activity and they are employed as natural antioxidants to control degenerative diseases.

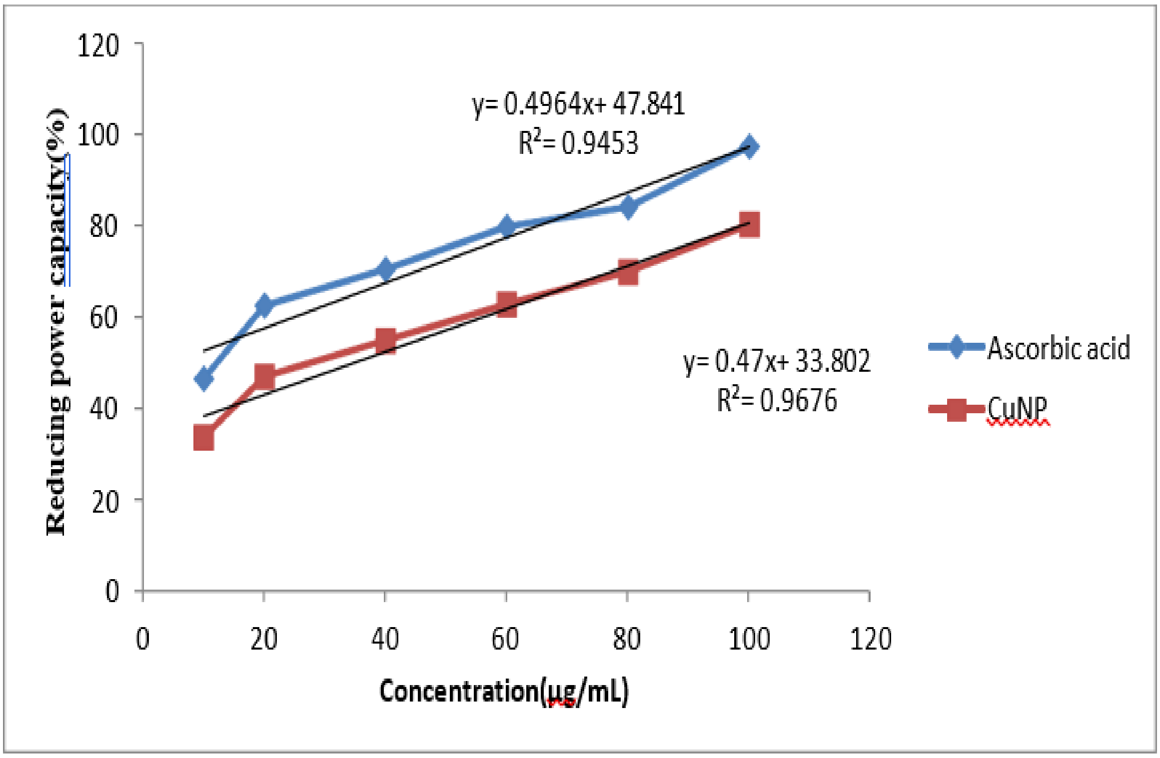
Ferric Reducing Antioxidant Power
Reactive oxygen species (ROS) has an important role in cell signaling while it can also lead to cellular oxidative damage which is the detrimental by products of cellular respiration. ROS are highly active on account of their unpaired valence shell electrons being free radicals besides being molecules and ions. Excess of ROS in the body leads to many diseases such as cancer, aging, cataracts, diabetes mellitus, cardiovascular disease and brain dysfunction. Due to the radical scavenging power, antioxidants play a significant role in terminating free radicals before they attack cells and biological targets thereby preventing various diseases.
FRAP assay is based on reduction of colourless Fe3+- 2,4,6-tripyridyl-s-triazine complex into Fe2+- 2,4,6 tripyridyl-s-triazine complex in the acidic medium. In order to identify reductive ability of CuO NPs, transformation of the Fe3+ - Fe2+was observed in the presence of CuONPs. The IC50 value of the ascorbic acid and biosynthesized CuONPs was found to be 4.35µg/mLand34.47µg/mL respectively (Table2;Fig.4b). The biosynthesized CuO NPs possess effective reducing power when compared to the standard ascorbic acid. This might be due to the presence of high phenolic concentration in the leaf extract.The concentration of CuONP was increased leads to increase in the reducing ability of CuONPs. The results of green synthesized CuO nanoparticles using Abutlion indicum leaf extract. Antimicrobial and photocatalytic dye degradation activities correlates with results of present study (Faheem Ijaz et al., 2017).

Minimum Inhibitory Concentration
Minimum Inhibitory Concentration is considered as standard procedure for determining the susceptibility of microorganism to various antimicrobials (Parvekar et al., 2020).The minimum inhibitory concentration needed for commercial antibiotic amoxicillin against the test organisms Escherichia coli, Staphylococcus aureus, Streptococcus sp, Bacillus subtilis and Enterococcus faecalis was 5.61µg/mL, 4.28 µg/mL, 1.14 µg/mL, 3.26 µg/mL and 59.14 µg/mL (Table 3). The minimum inhibitory concentration required for synthesized CuO NPs to inhibit the growth of test organism Escherichia coli, Staphylococcus aureus, Streptococcus sp, Bacillus subtilis and Enterococcus faecalis was 29.01 µg/mL, 47.34 µg/mL, 55.47 µg/mL, 35.83 µg/mL and 116.29 µg/mL (Table 3). The biosynthesized CuONPs exhibited moderate antibacterial activity in a dose dependent manner when compared to control. Further it was observed that, the antibacterial activity of CuO nanoparticles was effective against gram negative organisms (Fig. 5).

This result corroborates with other results which explain that antibacterial activity of CuONPs increases with an increased in concentration (Andualem et al., 2020; Amin et al., 2021). In contrast, Andualem et al., (2020) observed that CuONPs was more activeagainstgramnegativebacterialcells. Thisreportisinaccordancewith results of the present study. Further, comparable zone of bacterial growth inhibition and MIC of various biosynthesized copper nanoparticles was reported in many studies (Arya et al., 2018; Hassanien et al., 2018; Wu, 2022). The antibacterial activity of the phytosynthesized CuO NPs may also relates with the overall positive zeta potential charge. Bacterial cell membranes possess a negative charge in the surface that great attracts positively charged materials (Ssekatawa et al., 2020). Due to this electrostatic interaction, adsorption, consequent penetration of CuONPs into bacterial cells is easily possible thereby disturbing its cellular membrane and inhibit antibacterial activity. In addition Qamar et al., (2020) report that large rod shaped biosynthesized copper nanoparticles with a net charge of −7.3 mV and average size of 62 nm exhibit potent antimicrobial activity against gram positive and gram negative bacteria. This may explain that rod shaped nanoparticles has large surface area to volume ratio which enhances their interaction with the bacterial cells, thereby inhibiting their growth. These findings are clearly reveals that several parameters may influence the antibacterial activity of the synthesized nanoparticles. Further, the biosynthesized inorganic nanoparticles with a wide range of morphology also exhibits excellent antimicrobial activity as each morphological character endow with unique mechanism of bactericidal action.

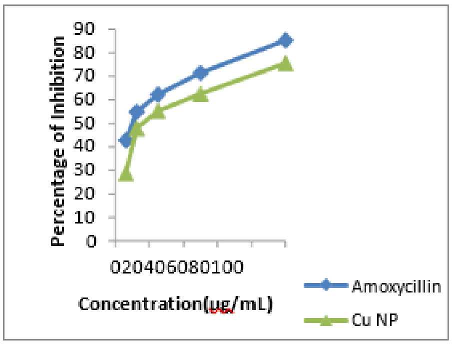


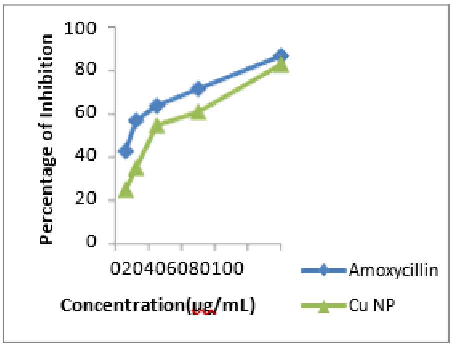
(a)



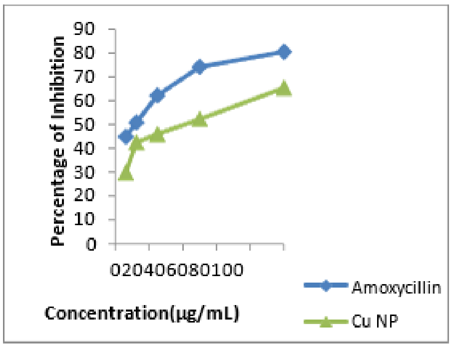
(b)

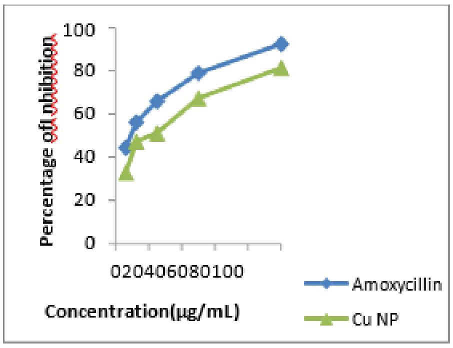
Fig.4.Antioxidant potential of biosynthesized copper oxide nanoparticles using aqueous leaf extract of Marynia annua
(a) DPPH free radical scavenging activity
(b)Ferric reducing antioxidant power activity



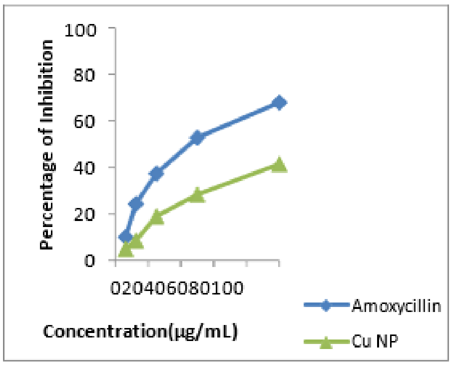


Escherichia coli *Bacillus subtilis*





Streptococcus aureus *Staphylococcus sp*



Enterococcus faecalis

Fig. 5. Minimum Inhibitory Concentration effect of biosynthesized copper oxide nanoparticles using aqueous leaf extract of Martynia annua leaf extract against wound pathogens.

Table1.DPPH free radical scavenging ability of synthesized copper oxide nanoparticles using aqueous leaf extract of Martynia annua.

S. No	Concentration (µg/mL)	Free radical scavenging ability of ascorbic acid (%)	Free radical scavenging ability of CuO NP (%)
1	10	36.16± 0.27	14.29± 0.42
2	20	47.73± 0.18	25.37± 0.63
3	40	55.43± 0.73	39.48± 0.29
4	60	64.27± 0.58	52.63± 0.76

5	80	73.19± 0.39	60.91± 0.14
6	100	80.45± 0.63	71.42± 0.45
7	200	93.57± 0.23	75.36± 0.59
IC50(µg/mL)		22.69± 0.43	77.70±0.46

Table2. Ferric reducing antioxidant power (FRAP) ability of synthesized copper oxide nanoparticles using aqueous leaf extracts of Martynia annua.

S. No	Concentration (µg/mlL)	Free radical scavenging ability of ascorbic acid (%)	Free radical scavenging ability of CuO NP (%)
1	10	46.49± 0.13	33.68± 0.43
2	20	62.52± 0.42	46.95± 0.25
3	40	70.49± 0.36	54.76± 0.63
4	60	79.84± 0.17	62.83± 0.54
5	80	84.16± 0.27	69.96± 0.25
6	100	97.43± 0.72	80.32± 0.72
IC50(µg/mL)		4.35±0.34	34.46±0.47

Table 3. Minimum Inhibitory Concentration (MIC) of synthesized copper oxide nanoparticles and amoxicillin antibiotic against tested wound pathogens.

Concentration (µg/mL)	Percentage of Inhibition									
	Escherichia coli		***Streptococcus sp***		***Staphylococcus aureus***		***Bacillus subtilis***		***Enterococcus faecalis***	
	Std	**CuO NP**	**Std**	**CuO NP**	**Std**	**CuO NP**	**Std**	**CuO NP**	**Std**	**CuO NP**
6.25	42.84	28.75	44.89	29.82	44.5	29.82	42.79	24.92	10.11	4.97
12.5	54.88	47.89	50.89	42.55	56.23	42.55	56.99	35.01	24.33	8.51
25	62.25	55.23	62.24	46	65.89	46	63.74	54.66	37.41	18.97
50	71.37	62.56	74.22	52.28	78.97	52.28	71.58	60.86	52.78	28.35
100	85.26	75.54	80.55	65.46	92.42	65.46	86.69	82.76	67.94	41.49
MIC(µg/mL)	5.61	28.01	4.28	47.34	1.14	37.34	3.26	35.83	59.14	116.29

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