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Biosynthesis of Manganese Nanoparticles (MnNPs) from *Brassica oleraceae* (Cabbage leaves) and its Antibacterial Activity

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Authors' contributions

This work was carried out in collaboration between both authors. Author SPA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author SS managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Manganese nanoparticles are promising material for various applications such as water remediation, catalytic oxidation reactions, bio sensors, etc for their superior adsorbing, electrochemical, catalytic, magnetic, supercapactive like properties additionally, MnNPs possess significant medicinal values, hence, this research aims at synthesizing MnNPs biogenically, characterize them to explore antibacterial activity. Aqueous cabbage extract was allowed to react with precursor $KMnO_4$ solution to synthesize the MnNPs. Then, MnNPs were characterized by: visual observation, UV-visible spectroscopy (UV-vis), X-ray diffraction (XRD), fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), and energy dispersive spectroscopy (EDX). Finally, agarwell diffusion was employed to study antibacterial activity against human pathogenic bacteria: *staphylococcus aureus* ATCC 25923, *escherichia coli* ATCC 25922 and *salmonella typhi*. Turning of purple color solution to reddish brown after stirring was the indication of the formation of nanoparticles. The formation of MnNPs was confirmed by appearance of sharp peak at 420 nm, the

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assigned band for MnNPs and the energy absorption band at 6 keV in EDX spectrum. Diffraction pattern of MnNPs revealed polycrystalline type with crystallite size of 10.70 nm. FT-IR result showed the chief biomolecules: flavoniods, alkaloids, proteins present in the extract which acts as reducing agent. FE-SEM confirmed the formation of spherical and ellipsoidal shaped agglomerated MnNPs. Agarwell diffusion method showed zone of inhibition (ZOI) of range 10-13 mm with (gram + ve and gram –ve) bacteria. Cabbage mediated biosynthesis of MnNPs is found simple, ecofriendly. This work revealed the formation of agglomerated MnNPs confirmed from uv-vis spectra and FE-SEM. Chief biomolecules of the extract act as reducing agent and stabilizing agent. Results of agarwell diffusion method depicted MnNPs are promising material for its antibacterial properties.

Keywords: Brassica oleraceae; manganese; nanoparticle; agarwell diffusion; antibacterial effect etc.

1. INTRODUCTION

Metal and their oxide nanoparticles have been remained to be one of the promising materials for having wide scope of applications such as optoelectronics, magnetic, catalytic, biosensor, super conductors, medicinal device, drug delivery and many more [1-5]. Usually, noble metals like gold, silver, platinum palladium nanoparticles have been given first priority for their superior medicinal properties [5-8] like for cancer treatment, targeted drug delivery, gene therapy, even DNA analysis [7 - 10] *etc*.. But, noble metal NPs are being highly expensive, other semi conductor metals like Cu, Zn, Fe, Mn nanoparticles have been started in use extensively [11-18].

Fabrication of Manganese (Mn) and its oxide NPs is one of the flourished topics in nanotechnology. Their superior adsorbing, electrochemical, catalytic, magnetic, supercapactive like properties etc [4,19] made them a high valued material and are found promising material for various applications such as water remediation, catalytic oxidation reactions, bio sensors, energy storage, magnetic resonance imaging and lithium ion batteries, medicines, drug delivery etc [20,21,23]. Mn and MnO NPs have been synthesized by several researchers for different properties like catalytic behavior, dielectric property, electrochemical property *etc* [20,21,24].

Over the past few years, biosynthetic route has been referred to as the efficient and novel technique for synthesis of nanomaterial. Generally, biosynthesis involves biological materials such as leaves, stems, roots, fruits, seeds, barks and various microbes like bacteria, fungi, yeast *etc*. The precursor salt particular metal ions are assumed to be reduced by various non toxic, naturally available biomolecules like, flavonoids, alkaloids, saponins, steroids, tannins, terpenes, etc. [25-27].

Different types of metal and their oxide NPs have been continuously synthesized using various plants such as: Copper nanoparticles (CuNPs) from *Mitragyna aryifolia Bark* [28], *Psidium guava L.* [25], *Ocimum Sanctum* leaf [29], *Gloriosa superb* [30], *Phaseolus vulgaris* [31], Silver nanoparticles (AgNPs) from banana peel [32], *Limonia acidissima* leaves and barks [33], Palladium (Pd) NPs from *Origanum vulgare L*. [34] *etc*. Similarly, microbe mediated biosynthesis through extracellular and intercellular mechanism has also been in use as novel protocol for fabricating NPs [17,35]. Various kinds of microbes such as Bacillus, Pseudomo-nas, Klebsiella, Escherichia, Enterobacter, Aeromonas, Corynebacterium, Lactobacillus, Pseudomonas, Weissella, Rhodobacter, Rhodococcus, Brevibacterium, Streptomyces, Tricho-derma, Desulfovibrio, Sargassum, Shewanella, Plectonemaboryanum, Rhodopseudomonas, Pyrobaculum, are used extensively in the synthesis etc. [36] Biogenically synthesized AgNPs [37], TiO_2 NPs [38], ZnO [39] are reported to be promising for biomedicine.

Cabbage [*Brassica oleraceae*] belonging to the family *Cruciferae,* has been used in the treatment of gastrointestinal disorders, minor injuries wounds, mastis, oxidative stress of cancer and coronary artery diseases from the very beginning . Essentially, medicinal behavior of the cabbage leaves attributes for its antioxidant effect, anti-inflammatory and antibacterial properties [40]. And the characteristic medicinal properties of cabbage leaves are due to the availability of phyto-constituents such as vitamin C, carotenoids, phenols, polyphenols, flavonoids, proteins and glucosinolates in the cabbage leaves [41].

Literature review revealed that the research over biosynthesized MnNP for antibacterial properties are scarce till date hence, this research is mainly focused on fabricating MnNPs biogenically using cabbage leaves extract to explore antibacterial activity.

2. METHODOLOGY

2.1 Preparation of Aqueous Cabbage Extract

At first, cabbage leaves were washed 2-3 times with distilled water so as to remove dust particles which were then grinded to form the paste. After that, 10 g of cabbage paste was heated along with 100 mL distilled water over water bath for half an hour. Resulting yellowish coloured solution was allowed to cool for some time and filtered through muslin cloth. The filtered aqueous cabbage solution was stored at 4ºC and used as the natural reducer and stabilizer for synthesis. 90 mL of extract was produced.

2.2 Biosynthesis of Manganese Nanoparticles

Bio-synthesis was carried out reacting 1 mL of aqueous cabbage extract and 10 mL of 1 mM $KMnO_4$ solution (1:10) following protocol with slight change [12]. The solution mixture was allowed to heat continuously at 40-45°C over magnetic stirrer till the colour change from purple to reddish brown as the colour change indicates the reduction of the salt to zerovalent metals [8, 18]. Then, the solution mixture was subjected to centrifugation at 3500 rps for complete separation of the solid residue. Finally, purification of the NPs was carried by repeating washing and centrifugation process. The collected NPs were oven dried at 40°C for 24 hours. The synthesis was further carried out varying the volumes of the extract, and at different time intervals to study their influence over biosynthesis. Snaps of biosynthesis process are shown in Fig. 1. The weight of as-synthesized MnNPs was 6.0 mg.

2.3 Characterization Techniques

Biosynthesized MnNPs were analyzed preliminarily simply by colour change and confirmed by monitoring UV–vis absorption band using double beam UV-visible spectrophotometer Model LT-2802 in the wavelength range of 300-800 nm at interval of 5 nm (Brand and Model). For investigation of phase morphology of the sample, Powder XRD BUKER D2 having CuKα (λ=1.54 A°) radiation was used in 2Θ angle at range of 10° to 80°. Similarly, functional groups associated with biomolecules were characterized using Fourier Transform Infrared Spectroscopy (FT-IR) Tracer 100 with a resolution of 4 cm^{-1} in ATR mode at the range of 500 – 4000cm^{-1}. The elemental composition was analyzed using the Energy Dispersive X-ray Spectroscopy of model EDX -8000. The surface morphology of the sample was analyzed using Field Emission Scanning Electron Microscopy (FE-SEM), Carl Zeiss, SUPRA 40 VP, Oberkochen, Germany. Antibacterial activity was explored using agar well diffusion method against Gram +ve and Gram -ve bacteria: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Salmonella typhi* [12].

3. RESULTS AND DISCUSSION

3.1 Visual Observation

Upon visual observation, the purple colour of the ($KMnO_4$) solution was turned to reddish brown after stirring it with aqueous cabbage extract for 20 minutes which was the indication of bio-reduction of Mn^{+7} to Mn^0. The reduction is completely biogenic as phyto-constituents of the cabbage played crucial role for reduction and stabilization of the NPs and the colour change owes to the excitation of electrons present at the surface of as-synthesized NPs [16,25].

3.2 UV-visible Spectroscopic Studies at Volume Variations

UV-vis spectroscopic technique is one of the reliable techniques for analyzing size and shape of NPs [11]. UV-vis spectra of as-synthesized NPs formed at different volume ratio of aqueous extract and the precursor salt ($KMnO_4$) solution such as 1:1; 1:3; 1:5 &1:10 are presented in Fig. 2.

The Fig. 2 glimpses distinct UV-vis absorption bands in all of the volume ratios with appreciable difference in position and intensities which may be due to formation of different shape and sized NPs. It is well acquainted that the absorption band attributes the Surface Plasmon Resonance (SPR) of electrons present at the surface of NPs [8,18]. The nature of UV-vis absorption band (Fig. 2) formed by ratio 1:1 & 1:3 at 380 nm is of broad type indicating formation of agglomerated type of NPs. Comparatively, the absorption bands of 1:5 & 1:10 are less broad with that of other samples and observed at 425 and 420 nm,

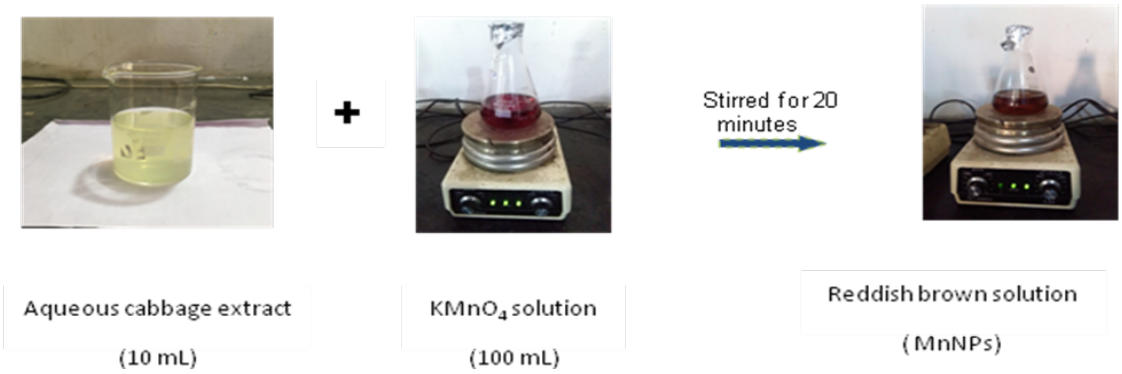

Fig. 1. Biosynthesis of Manganese (Mn) nanoparticles using cabbage leaves extract and 1 mM $KMnO_4$ at volume ratio (1:10)

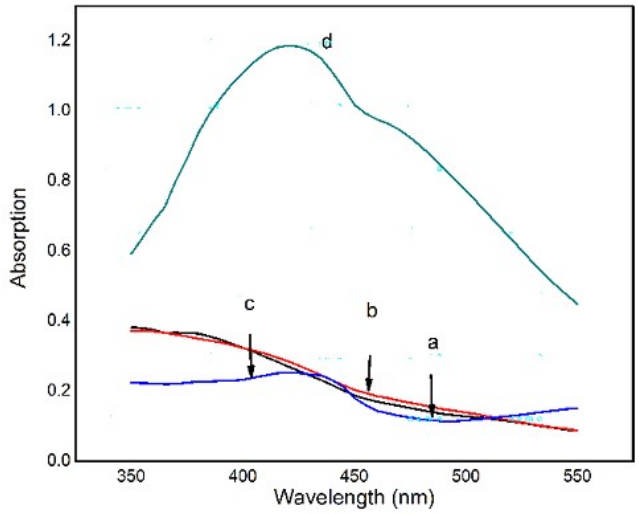

Fig. 2. UV-visible spectra of Manganese nanoparticles (MnNPs) using aqueous cabbage extract and $KMnO_4$ at different volume ratio: a) 1:1, b) 1:3, c) 1:5 & d) 1:10

respectively which are the assigned band of MnNPs [2]. At the same time, the intensity of absorption band at 1:10 ratio is higher than the rest of the samples which is due to the formation of considerable amount of smaller sized NPs as revealed by the literatures [42,43]. An appreciable amount of MnNPs formed by 1:10 volumes are due to higher rate of nucleation of Mn^{+7} ions as suggested by Carollling et al. [25]. Hence, the UV-vis result shows that the ratio 1:10 is found to be the optimum volumes for the biosynthesis of MnNPs. Further, the UV-vis absorption band shown by 1:10 volumes is multiple peak indicating formation NPs of different shapes like spherical and ellipsoidal [2,43].

3.3 UV-visible Spectroscopic Studies with Time Variations

The Fig. 3 is the UV–vis spectra of aqueous cabbage extract, and MnNPs (1:10) formed at various time intervals: 5, 10, 15, 20, 25 and 30 minutes. In the figure, UV-vis absorption band of cabbage extract doesn't contain any distinct band while all the other curves show the unique UV-vis absorption band *i.e* 420 nm except in 30 minutes sample depicting that MnNPs were fabricated conveniently using the extract. The UV-vis absorption band is broad type revealing the formation of aggregated MnNPs and display red shift after 25 minutes attributing the presence of bigger size of MnNPs; this result is similar to that of literature [2] which disclosed about the red

shift of UV-vis absorption bands with increase of time used for biosynthesis. At the same time, the less intense peak at 25 minute shows that only small amount of MnNPs were fabricated compared to other samples.

3.4`X-Ray Diffraction (XRD) Spectroscopic Studies

The phase structure of bio-synthesized MnNPs was explored *via* powder X-ray diffraction. The diffraction pattern of as-synthesized MnNPs (Fig. 4) consists of sharp peaks at 26.0°, 34.0°, 36.6°, 53° and 57° indicating poly crystalline type. Additionally, some less intense peaks observed at 28°, 38°, 53°, 57° and 60° infirmed to the formation of manganese oxide as suggested by [20]. The size of the crystallite was calculated using Debye Scherrer equation (Equation no. 1) at intense peak (26.2765°) with full width at half maximum (FWHM) value 0.7620 which was found to be 10.70 nm.

$$D = {0.9 \times \lambda}/{\beta \mathrm{Cos}\Theta} \quad (1)$$

Where, λ is the wavelength of the source used, β is full width at half maximum (FWHM), Θ is the diffraction angle [25,38]. Appearance of some noises revealed the presence of some impurities which may be due to lack of heating.

3.5 Fourier Transform Infrared Spectroscopic Studies

FT-IR spectrum of biosynthesized MnNPs showed presence of bands at 3234, 2336, 2090 and 1634 cm^{-1} in Fig. 5 corresponding to -O-H stretching of H-bonded alcohol and phenols, alkyne, -C-O, >CO, and –N-H groups, respectively [28,30,43]. It was revealed that O-H stretching of alcohols, phenols at 3400 cm^{-1} by [11]. However, the band of -O-H stretching is slightly shifted towards lower wavelength indicating that the organic molecules are associated with MnNPs. Consequently, FT-IR result showed as-synthesized MnNPs are stabilized by flavoniods, alkaloids, proteins present in the extract.

3.6 Energy Dispersive-ray (EDX) Spectroscopy

EDX spectrum (Fig. 6) shows the existence of energy absorption band at 6keV which is the characteristic band of Manganese (Mn) thus; it confirmed the presence of elemental Mn. Further, the energy absorption band of Mn reported here is supported by [43].

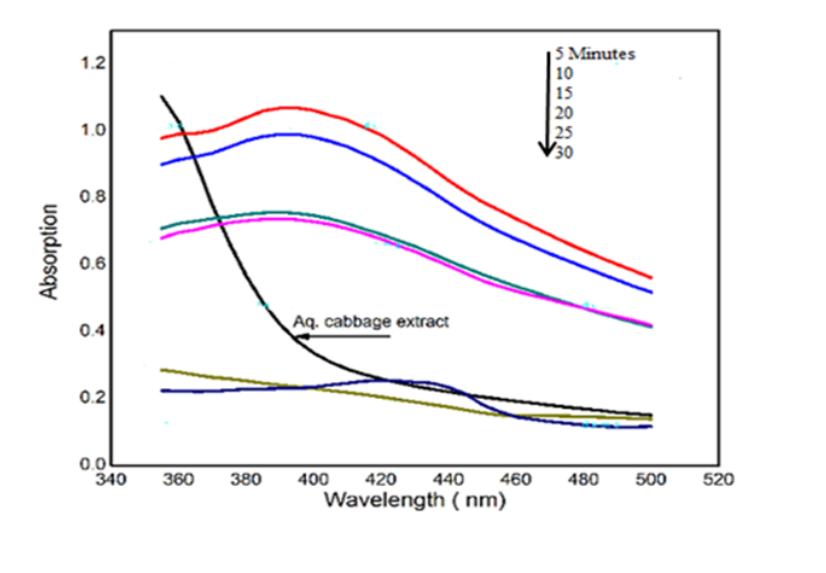

Fig. 3. UV-vis spectra of aqueous cabbage extract and MnNPs of ratio 1:10 at various time intervals

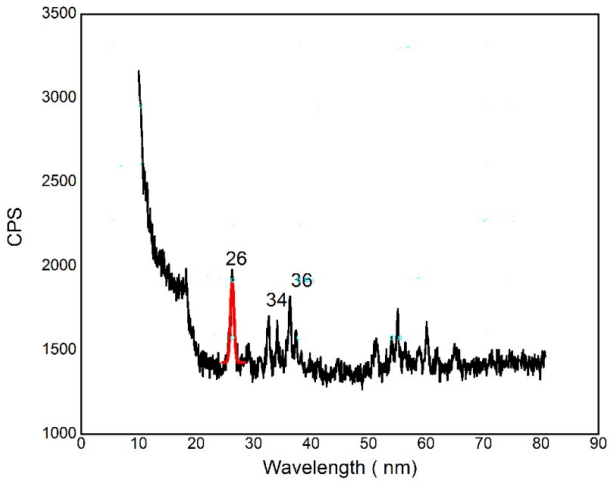

Fig. 4. Diffraction pattern of MnNPs synthesized using cabbage leave extract and $KMnO_4$ solution (1:10) at 40°C

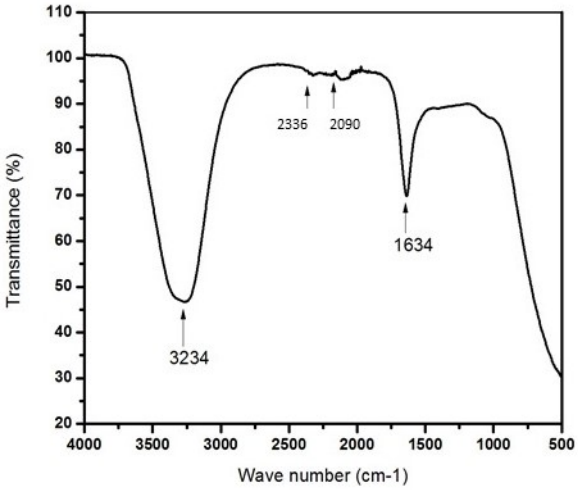

Fig. 5. Fourier Transform Infrared (FTIR) spectra of biosynthesized MnNPs using $KMnO_4$ and cabbage extract (1:10)

Additionally, the EDX spectrum revealed presence of Mn of 36.08% by weight (Table 1). Energy absorption bands at 2, 3, 8 and 20 KeV corresponds to Si, K, Cu and Rh, respectively which were supposed to be formed during sampling process.

3.7 Field Emission Scanning Electron Microscopy (FE-SEM)

FE-SEM images of MnNPs are shown in Fig. 7(a-b). The figures clearly revealed the presence of agglomerated NPs of spherical and ellipsoidal shaped which are in support with that of UV–vis spectroscopy. Likewise, the SEM image at higher magnification showed aggregated MnNPs of spherical shape.

3.8 Antibacterial Activity

The result obtained from agar well diffusion method is presented in Fig. 8. The figure clearly showed zone of inhibitions (ZOI) at different concentrations of bacterial suspensions against each type of bacteria *i.e*, Gram +ve (*S. aureus*) and Gram –ve (*E. coli* & *S. typhi*).

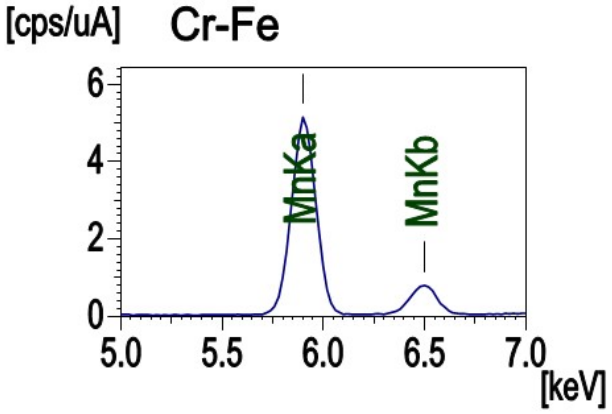

Fig. 6. Energy dispersive X-ray spectrum (EDX) of as synthesized Manganese nanoparticles

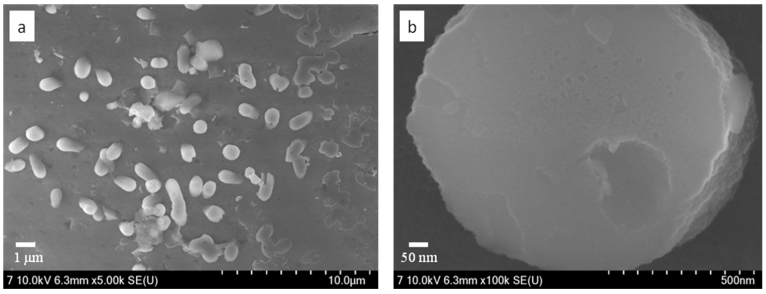

Fig. 7. Field Emission Scanning Electron Microscopic image (SEM) of bio-synthesized MnNPs (1:10), at (a) lower magnification and (b) higher magnification

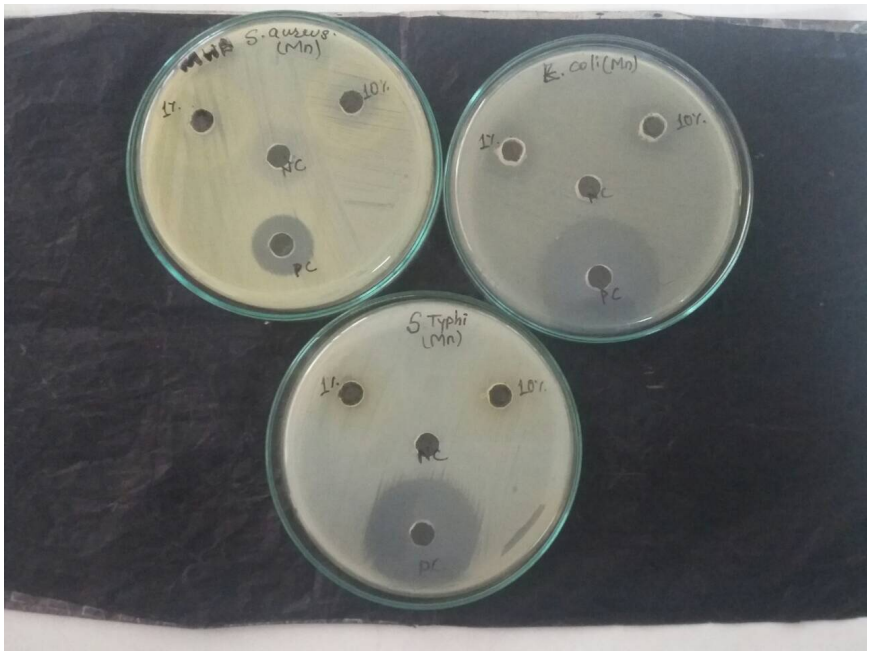

Fig. 8. Antibacterial activity of MnNPs against different bacteria (Gram +ve) *Staphyllococcus aureus Salmonella typhimurium*, and *Esherichia coli* (Gram –ve)

From the measurement, the ZOI so achieved was not so significant which was due to the agglomerated MnNPs. The measured values of ZOI of MnNPs are presented in Table 1.

ZOI produced by 1% and 10% diluted suspensions of MnNPs against both types of bacteria range from 10 – 13 mm. The result seemed supported by various biogenically synthesized metals or its oxide NPs [16]. From the literatures, metal or its oxide particles of <100 nm size can readily enter bacterial cell membrane having bigger pore size, and interact with bacterial cells resulting into drastic changes in physiological processes, damaging bacterial cell. Further, bacterial cell death is attributed to electrostatic interaction between electro positive MnNPs and electro negative cell membrane surface [10-11]. Thus, agar well diffusion method inferred that biosynthesized MnNPs are potential nanomaterial for biomedicine.

Table 1. Zone of inhibition shown by Ofloxacin and MnNPs of different concentrations towards Gr +ve, Gr –ve bacteria

Test organisms	Zone of inhibition (ZOI)		
	MnNPs		Ofloxacin
	1%	10%	
Staphylococcus aureus	10 mm	13 mm	17 mm
Salmonella typhi	10 mm	10 mm	33 mm
Escherichia coli	12 mm	12 mm	32 mm

4. CONCLUSION AND RECOMMENDATIONS

Biosynthesis of manganese nanoparticle (MnNPs) was conveniently carried out using non toxic aqueous cabbage extract at laboratory condition. The confirmation of MnNPs was achieved from the characteristic UV-visible absorption band at 420 nm and distinct energy absorption band at the region of manganese in EDX spectrum. Quantitatively, the sample consists of 38 % Mn by weight. The synthetic process was environmentally benign as the MnNPs were fabricated simply by reducing behavior of various organic molecules comprising phenolic, amines and carbonyl group which was inferred from FT-IR analysis. Phase structure was characterized by XRD and the crystallite size of MnNPs was calculated to be 10.70 nm. FE-SEM image revealed the presence of agglomerated MnNPs of spherical and ellipsoidal shaped nanoparticles. Finally, agarwell diffusion method showed that MnNPs have mild antibacterial effect against Gram +ve and Gram –ve bacteria. Overall, the process is concluded to be fast, cost effective and environmentally benign. Exploration of catalytic properties, effect of other physicochemical parameters on synthesis of MnNPs is highly recommended for future studies.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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