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Short Communication

Evaluation of ZnS:Mn Nanoparticles Capped with *Aloe vera* Gel Protein in Drug Delivery System

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Abstract

Objective: This study is aimed to find out the in vitro antimicrobial activity *Aloe vera* gel protein encapsulated Mn^{2+} doped ZnS nanoparticles (NPs) for drug delivery, biological, phytochemical and molecular properties of *Aloe vera* leaf gel extracts.

Methods: The protein is extracted from *Aloe* leaf gel by trichloroacetic acid-acetone method and tannins, saponins, flavonoids and carbohydrates were qualitatively analysed. ZnS:Mn nanostructured particles were prepared by chemical precipitation method. 25mL of each $Zn(CH_3COO)_2$, $MnCl_2$ and Na_2S solutions in water were used for preparation of Mn^{2+} doped ZnS NPs. Antibacterial assay of the ZnS:Mn nanostructured particles encapsulated with *Aloe vera* gel protein (ZnS:Mn/AV) was done by the well-diffusion method. And the genomic DNA of fresh and dried *Aloe vera* leaf gel was done by using cetyltrimethyl ammonium bromide method.

Results: Antibacterial activity of *Aloe vera* gel protein (ZnS:Mn/AV) is significantly above that of uncapped nanoparticle (NP) and gentamycin. The presence of tannins, saponins, flavonoids, carbohydrates was detected using standard protocols. Fresh young and dried leaf gel was used for DNA isolation and yielded good quality DNA.

Conclusion: During this study, protein extracted from *Aloe vera* (L) Burm. f (synonym *Aloe barbadensis* Miller) is employed as a capping agent to change the NPs widely exploited medicinal plants that have vast properties in the field of medicines. We report the antibacterial activity of ZnS:Mn NP encapsulated with *Aloe vera* gel protein synthesized by the chemical precipitation method, qualitative analysis of phytochemicals in *Aloe vera* gel extracts and isolation of genomic DNA from the *Aloe vera* plant. The ZnS:Mn capped with *Aloe vera* gel protein were also compared with the uncapped ZnS:Mn NP.

Keywords: capping, *Aloe vera*, nanoparticles, antibacterial activity

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1 INTRODUCTION

Over the years, preparing nanoparticles (NPs) for drug delivery is assumed to be the significant research area in new approaches of drug delivery systems. The most favoured feature of drug NPs is their biocompatibility in order that they will adapt to the body of the living organisms without causing damages. These days it's evidenced that the drug delivery systems supported NPs are more acceptable and have less side effects. Therefore, visible of this science it's vital to form use of the benefits of this science to supply novel drugs. Nanotechnology is a recent field of science which plays an important role in day today life aspects, creating great enthusiasm within the vast arena of life sciences especially biomedical devices and pharmaceutical field. Using noble metals such as gold, silver, platinum and zinc NPs can be synthesised and these are widely applied in products like tooth paste, detergents, cosmetics etc. that directly in contact with the human body.

A vast number of techniques are adopted to cap the surfaces of NPs with organic substances or polymers to arrest the growth of NPs and later stabilize them from agglomeration. Usually, chemical polymers are used to cap the surface of the NP and to create them more eco-friendly. There is an accepted green method which contains the biomolecules like proteins, amino acids, enzymes, vitamins, phenolics, saponins, tannins, alkaloids, and terpenoids, present in plant extracts, for reduction and stabilization of metal ions. A number of these biomolecules act as electron shuttles in metal reduction, while other constituents are liable for capping of resulting NPs, which regulates the aggregation of NPs and also in post surface modification of NPs. Within the present work protein extracted from *Aloe* is employed as a capping agent to stabilize ZnS:Mn NP and it's produced by simple chemical precipitation method[1]. It aims to analyse its antibacterial activity and comparing it with the antibacterial activity of uncapped ZnS:Mn NP. Phytochemical analysis of *Aloe* gel and isolation of genomic DNA is also performed in the present study.

2 MATERIALS AND METHODS

2.1 Protein Extraction by Trichloroacetic Acid-acetone Method

The leaf gel of thick tender leaves of *Aloe vera* was cut into small pieces[2]. Grind sample (leaf gel) into a fine powdered in a mortar and pestle using liquid nitrogen. The powder was mixed with 10% trichloroacetic acid/acetone and centrifuged. To this pellet 80% methanol and 0.1M ammonium acetate were added and centrifuged. The supernatant was thrown away and pellet was again mixed with 80% acetone and centrifuged. Discarded the supernatant and the pellet is air dried at room temperature to remove residual methanol[3]. About 0.1g dried pellet were added to 1:1 phenol (pH 8.0)/ sodium dodecyl sulfate buffer and mixed thoroughly and incubated the mixture for 5min and centrifuged. The upper phenol phase is then transferred into a new tube and stored at -20°C for 10min to overnight and centrifuged, discarded the supernatant; a white pellet is obtained. Washed the pellet once with 100% methanol and once with 80% acetone. Allow protein to air dry briefly and is dissolved in buffer of choice (e.g. sodium dodecyl sulfate sample buffer or isoelectric focusing rehydration buffer). Proteins were qualitatively analysed.

2.2 Chemical Precipitation Method

ZnS:Mn nanostructured particles were prepared by chemical precipitation method. 25mL of each $Zn(CH_3COO)_2$, $MnCl_2$ and Na_2S solutions in water were used for preparation of Mn^{2+} doped ZnS NPs. 0.02M solution of $MnCl_2$ was added drop wise to 1M $Zn(CH_3COO)_2$ solution and heated to 70°C. 0.02gms AV/mL water was prepared. Two different volume of prepared *Aloe vera* solution was taken; 2.5mL and 5mL respectively. And to these solutions 1M Na_2S solution were added simultaneously with continuous stirring using magnetic stirrer[4].

The solutions were stirred for 20min keeping temperature constant. The resulting white colloidal suspension was filtered, washed with de-ionized water and dried by keeping in an oven at 70°C for one day. The same method was also used for the preparation of uncapped sample[1].

2.3 Antibacterial Assay

Antibacterial assay of the ZnS:Mn nanostructured particles encapsulated with *Aloe vera* gel protein (ZnS:Mn/AV) was done by the well-diffusion method[5] against five pathogenic bacterial strains such as *Staphylococcus aureus* (MTCC 7443), *Escherichia coli* (MTCC 1687), *Pseudomonas aeruginosa (*MTCC 1688), and *Klebsiella pneumoniae* (MTCC 4030), *Proteus mirabilis sp.* These strains were cultured in a sterile nutrient broth for overnight and the thick inoculum is then lawn cultured on Mueller Hinton Agar plates for antibacterial assay. A gel puncher was used to make 4mm wells on the agar media, sufficiently separated from each other to avoid overlapping of inhibition zones. 0.1mg each of AV powder, ZnS:Mn NP and (ZnS:Mn/ AV) were added into the wells and incubated at 37°C in a bacteriological incubator. Gentamycin was used as

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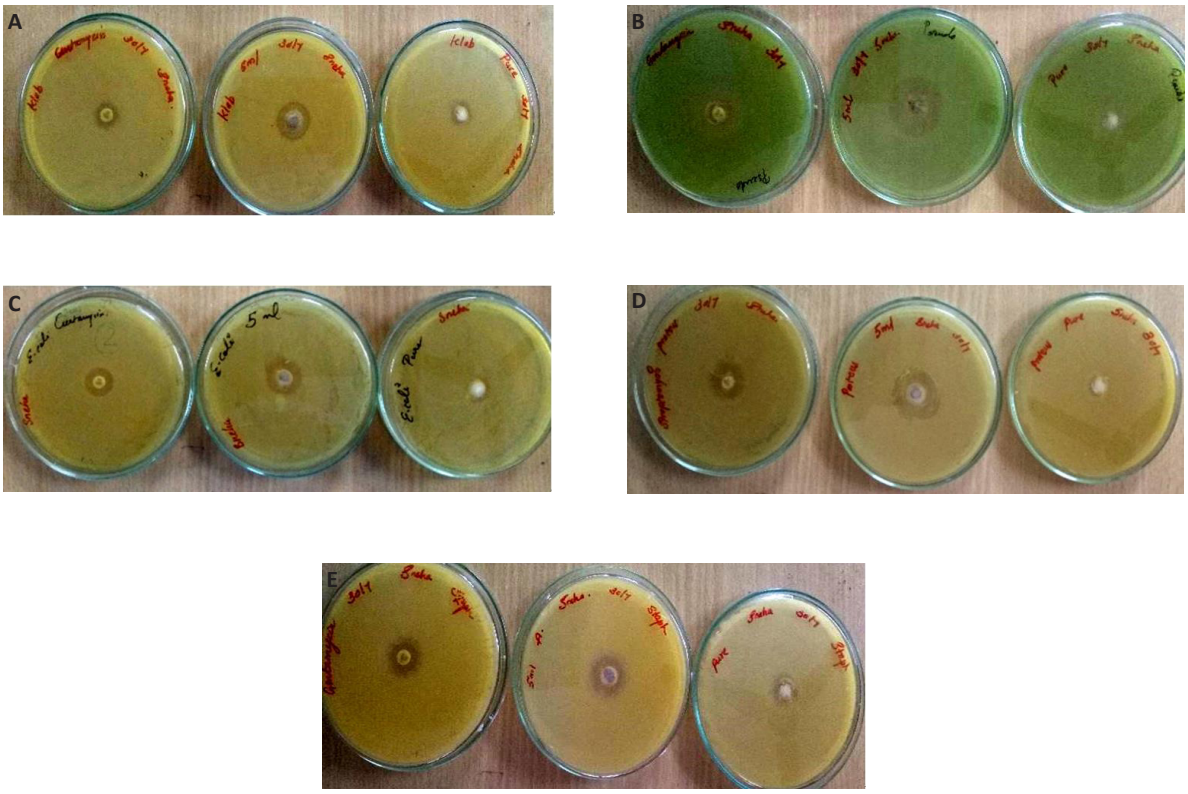

Figure 1. The zone of inhibition of *Klebsiella pneumoniae* (A), *Pseudomonas* (B), *Escherichia coli* (C), *Proteus* (D), and *Staphylococcus* (E) in Gentamycin, ZnS:Mn/AV, ZnS:Mn, respectively.

Table 1. Zones of Inhibition (mean±SD) Bacterial Strains against Three Different Substances

Bacterial Strain	ZnS:Mn/AV Nanoparticle (mm)	ZnS:Mn Nanoparticle (mm)	Gentamycin (mm)
Escherichia coli	20.5±0.50	14±0.25	14±0.30
Klebsiella pneumoniae	20±0.25	12.62±0.37	13.75±0.25
Proteus mirabilis	27.25±0.75	14.25±0.75	19.5±0.5
Staphylococcus aureus	19 .15±0.15	13.02±0.02	15±0.10
Pseudomonas aeruginosa	30.50±0.50	20.25±0.75	28.62±0.37

a positive control and NPs alone as a reference control. The inhibition zone is measured from edge to edge of the clear area in millimetres[1].

2.4 Phytochemical Analysis

The *Aloe vera* leaf gel was homogenized in a mortar and pestle and qualitative analysis of tannins, saponins, flavonoids and carbohydrates were qualitatively analysed.

2.5 Isolation of Genomic DNA

The genomic DNA was extracted using molecular biology grade cetyltrimethyl ammonium bromide (CTAB) as proposed by Doyle and Doyle et al[6].

3 RESULTS AND DISCUSSION

3.1 Antibacterial Assay

The results of antibacterial studies shows that ZnS:Mn nanostructured particles encapsulated with *Aloe vera* gel protein (ZnS:Mn/AV) supressed the bacterial growth and produced better zones of inhibition than ZnS:Mn uncapped NP and gentamycin (Figure 1 and Table 1). The NPs also showed fluorescence. It is noted that the nanostructured particles with *Aloe vera* gel protein (ZnS:Mn/AV) were very effective against pathogenic bacterial strains *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Proteus mirabilis* (Figure 2). The findings were tabulated and statistical analysis were done using two way analysis of variance and later validated the antibacterial activity of encapsulated with *Aloe vera* gel protein ZnS:Mn/AV. There are many reports that proves the use of *Aloe vera* gel for the treatment of various kinds of gastrointestinal irritations, to relieve thermal burn and sunburn, promote wound healing, and moisturize and soften skin[7]. *Aloe vera* also has got strong antibacterial,

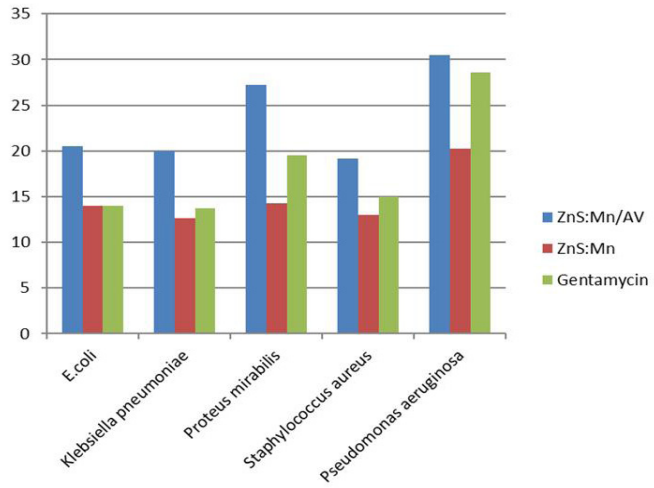

Figure 2. Graphical representation of Zone of inhibition of each bacterial strains with three different substances.

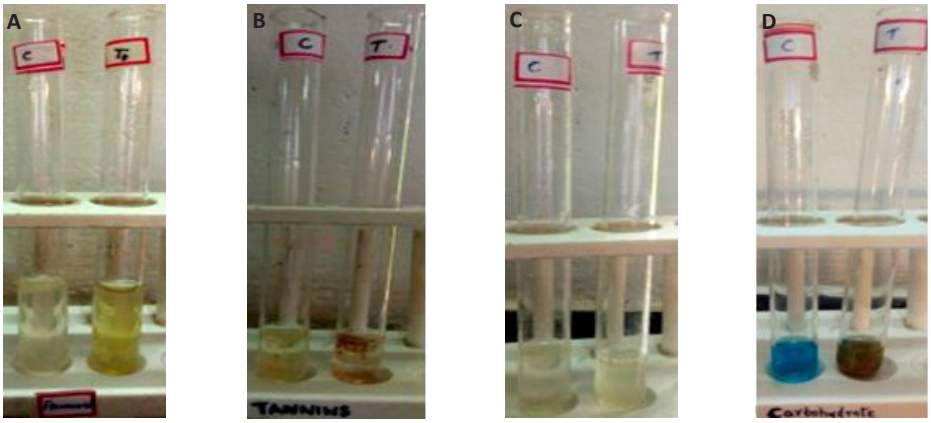

Figure 3. Flavonoid (A), tannin (B), saponins (C), and carbohydrate (D).

antifungal, and antiviral properties[8,9]. The encapsulated with *Aloe vera* gel protein (ZnS:Mn/AV) showed clear zone of inhibition against all the test organisms used in this study, validating the use of encapsulated with *Aloe vera* gel protein in drug delivery for better interaction between microbes and the human body.

3.2 Phytochemical Analysis

Phytochemical analysis of the *Aloe vera* gel extracts were done and presence of tannins, saponins, flavonoids, carbohydrates was identified (Figure 3).

3.3 Isolation of Genomic DNA

Genomic DNA isolation was done by CTAB method and isolated bands were visualized under UV transilluminator (Figure 4)

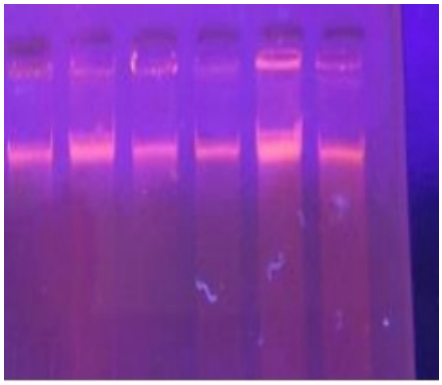

Figure 4. Genomic DNA of *Aloe vera* leaf gel.

4 CONCLUSION

In our conclusion the findings clearly demonstrated the antibacterial properties of the *Aloe vera* gel protein capped ZnS:Mn NPs. This could be used in biomedical applications such as drug delivery, bio-imaging, in cancer detection and in the field of development of antibiotics and for cosmetic treatments[2]. Presence of various phytochemicals were also determined which could be used for medical applications. Genomic DNA bands were obtained, which would be used for future applications. We also found that *Aloe vera* gel protein capped ZnS:Mn NPs ZnS:Mn/AV showed luminesce and further studies work will also investigate

luminescent property the of the material, its application in biomedical field[2]. Also, future work includes analysis of phytochemicals for their properties, and conjugation of these phytochemicals.

Acknowledgements
Not applicable.

Conflicts of Interest
All authors declared no conflict of interest.

Author Contribution
All authors have equally contributed towards this research manuscript. All authors approved the final version.

Abbreviation List
CTAB, Cetyltrimethyl ammonium bromide
NPs, Nanoparticles

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