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RESEARCH ARTICLE

ANTI-BACTERIAL ACTIVITY OF ZINC OXIDE NANOPARTICLES PREPARED FROM BRASSICA OLERACEAE LEAVES EXTRACT

AMRITA RAJ*, REENA SHIRLEY LAWRENCE,MOHAMMAD JALEES, KAPIL LAWRENCE

Department of Chemistry, SHIATS, Allahabd (U.P.), India

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***Corresponding Author**

AMRITA RAJ

Abstract

Present study focuses on the green synthesis of ZnO nanoparticles by zinc nitrate and utilization of bio components of leaves extract of *Brassica oleraceae.* The characterization of the nanoparticles was examined by UV-Vis spectrum and scanning electron microscope (SEM). We investigated the antibacterial activity of ZnO nanoparticles with various particle size (1-100nm). on gram positive and gram negative bacterial strains by agar well diffusion method and their MIC and MBC values were determined. Antibacterial activities against *E. coli, V. cholera, C. Botulinum, S.aureus, B. subtilis* were evaluated by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Results indicate that zinc oxide nanoparticles had strong antibacterial activity against *bacteria*. The activity increased as the concentration of the nanoparticles increased. The MIC and MBC were 50ug/ml and 25µg/ml, respectively. Our study indicates that zinc oxide nanoparticles could potentially be an antibacterial reagent to treat diseases caused by these bacteria.

INTRODUCTION

Nanotechnology is the ability to observe measure, manipulate, and manufacture things at the nanometer scale, the size of atoms and molecules. This science is technological innovation in the 21^{st} century. Research and development in this field is growing rapidly throughout the world. A major contribution of this field is the development of new materials in the nanometers scale. These are usually particulate materials with at least one dimension of less than 100 nanometers (nm), even the particles could be of zero dimension in the case of quantum dots. Metal nanoparticles have been of great interest due to their distinctive features such as catalytic, optical, magnetic and electrical properties. Nanoparticles exhibit completely new or improved properties with larger particles of the bulk materials and these novel properties are derived due to the variation in specific characteristics such as size, distribution and morphology of the particles. Nanoparticles present a higher surface area to volume ratio with decrease in the size, distribution and morphology of the particles[1]**.**

Green synthesis of nanoparticles has gained significant importance recent years and has become one of the most preferred methods. Green synthesis methods have several merits such as their simplicity, good stability, less time consumption, non-toxic byproducts and large scale synthesis. Extensive studies have been made on the green synthesis of nanoparticles of nobel metals like gold and silver[2]**.** The growing need of environmental friendly nanoparticles, researchers are using green methods for the synthesis of various metal nanoparticles for pharmaceutical application. Often chemical synthesis methods like sol-gel process, micelle, chemical precipitation, hydrothermal method, pyrolsis, chemical vapour deposition etc. lead to the presence of some toxic chemical species adsorbed on the surface that may have adverse effects in medical application[3]**.**

ZnO nanoparticles exhibits a high degree of cancer cell selectivity with the ability surpass the therapeutic indices of some commonly used chemotherapeutic agents. It is used in paints, sunscreens, plastic, rubber manufacturing electronics and pharmaceuticals products. It is also potentially used to treat leukemia and carcinoma cancer cell. It is also used as drug carrier, cosmetics and fillings in medical materials**.** However most ZnO nano-particles used commercially have significant advantages, compared to silver nano-particles, especially cost effectively[4]**.**

The antibacterial activity of ZnO nanoparticles (NPs) triggered by generation of reactive oxygen species (ROS) depending on the fate of photoexcited charge carriers. Batches of wide band gap ZnO NPs of 79nm sizes, capped with polyethylene glycol (PEG), ascorbic acid (AsA), mercaptoacetic acid (MAA) and polysorbate 80 (T80) were synthesized by precipitation method. These capped ZnO NPs exhibited ROS induced antibacterial activity, where the ROS was measured by TBARS assay. The PEG capped and AsA capped ZnO NPs exhibited weaker antibacterial activity and were correlated with strong and broad green emission peak owing to oxygen vacancies[5].

MATERIALS & METHOD:

Preparation of *Brassica oleraceae* leaf extract

The leaves of *Brassica oleraceae* were brought from the local market of Lucknow and identification was done by NBRI, Lucknow. The leaves were washed with distilled water to remove the dust particles and then dried in the absence of light. The dried leaves were cut and grinded for preparing powder.

Extract Preparation

Plant extract was prepared by mixing of 5g of plant powdered in 100ml of ethanol (95%) in a 250 ml conical flask. The solution was kept for 24 hours and then filtered using mesh. Further study has been carried out from this extract.

Green synthesis of ZnO Nanoparticles

The Leaves extract of *Brassica oleraceae* was used for reduction of Zinc ions (Zn^{2+}) to Zinc oxide nanoparticles. *Brassica oleracea* leaves extract was boiled to 60-80^{o}C and then Zinc Nitrate solution was added to the solution as the temperature reaches 60^{o}C. This solution was boiled until it become a pale- yellow colored solution and allowed to cool to obtain a transparent solution which indicates the formation of ZnO nanoparticles.

Characterization

The UV/Vis spectra were visualized by UV-Vis spectrophotometer (Systronics, Double Beam UV-VIS Spectrophotometer 2202) from CytoGene Research & Development, Lucknow. After the addition of zinc Nitrate to the plant extract, the spectra's were taken in different time intervals up to 24 hours between 350 nm to 410 nm.

Scanning Electron microscope analysis was done to observe the morphological features of synthesized zinc oxide nanoparticles from *Brassica oleraceae* leaves extract. Scanning Electron Microscope used was Electron Probe Micro Analyzer (JEOL MODEL No JXA8100) from Allahabad University, Allahabad.

Antibacterial activity

Gram positive (+) bacteria (*Escherichia coli and Vibrio cholera*) and Gram negative (-) bacteria (*Clostridium botulinum, Staphylococcus aureus* and *Bacillus subtilis)* have been used for the in-vitro antibacterial activity, the bacterial pathogens were collected from the Microbial culture collection bank of Department of microbiology and Fermentation Technology. SHIATS, Allahabad (U.P.). A test bacterium was grown and maintained on Nutrient agar slant at 37^{o}C. The organisms were sub-cultured once in every 15 days. Antibacterial activity of the generated Zinc nanoparticle was determined using agar well diffusion method elucidated by[6]. For determination of minimum inhibitory concentration (MIC), defined as the lowest concentration of material that inhibits the growth of an organism[7], was determined based on batch cultures containing varying concentration of nanoparticles in suspension (20–300 mg l^{-1}). Minimum Inhibitory Concentration (MIC) was determined by preparing the dilutions of the extract of *Brassica oleraceae* in sterile nutrient broth to achieve a decreasing concentration ranging from 100µl/ml to 50µl/ml.

RESULT & DISCUSSION:

Addition of leaves extract of *Brassica oleracea to 1mM* aqueous solution of Zinc nitrate change the colour of extract from pale yellow to colorless . The color change is due to excitation of surface plasmon vibration. The intensity of color increase with increase in time duration indicating the continuous reduction of Zinc ions. The formation of ZnO nanoparticle in the reaction mixture was confirmed by plasmon resonance of ZnO nanoparticles at 409 nm by UV-Vis spectrophotometer.

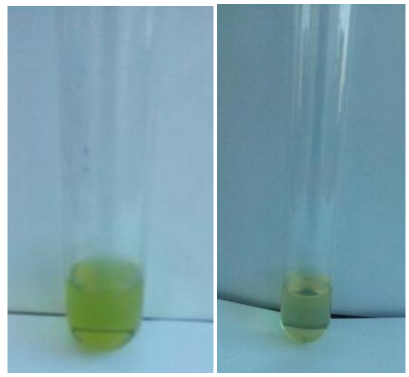
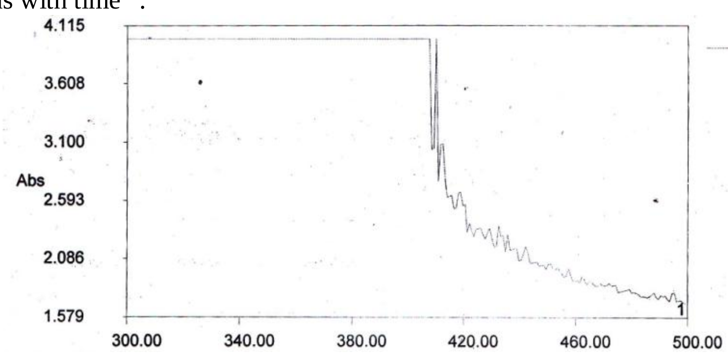


Figure1: Colour change indicating synthesis of ZnO nanoparticles Photographs of (A) After adding extract, pale- yellow color appear kept in 2 hours then heat at 60^0c then cool (B)transparent color appear.

The ethnobotanical plant *Brassica oleraceae* leaves has been reported to have, antiplasmodial, anticonvulsant, anti-bacterial, anti-oxidant, antifungal, hypolipidemic and free radical scavenging activities. This plant is said to be rare because of its seasonal habitat and sporadic distribution[8]**.**

Plants attributed the way to synthesize nanoparticles through green method because of their green chemistry principle.Biosynthesized silver nanoparticles using *Brassica oleraceae* and used these nanoparticles in antibacterial applications. Zinc oxide nanoparticles are very special among the existing nanomaterials due to their organometallic properties. Now it is possible to prepare individual nano metal or oxide particles. The whole plant body of *Brassica oleraceae* has evident to possess aphrodisiac activities and it has significant role in maintaining maleness. The Siddha medicine explains it as rejuvenating herb and it is known to possess coumarin, which is responsible for the hypolipidemic activity .We strongly believe that the phytochemicals, like coumarin are working as reducing agent and responsible for the conversion of metallic oxide into nanoparticlese[9].

Reduction of zinc ions into zinc oxide nanoparticles during exposure to plant extracts was observed as a result of the color change. The color change is due to the Surface Plasmon Resonance phenomenon. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The sharp bands of zinc oxide nanoparticles were observed around 384 nm. From different literatures it was found that the 409The absorption wavelength at about 300 nm of ZnO suggested the excitonic character of Zinc at room temperature. The UV emission is attributed to the radiative recombination between the electrons in the conduction band and the holes in the valence band while working on ZnO phosphor powders nm. So we confirmed that *Brassica oleraceae* leaf extract has more potential to reduce zinc oxide ions into zinc oxide nanoparticles which lead us for further research on synthesis of zinc oxide nanoparticles from *Brassica oleraceae* leaf extracts. The intensity of absorption peak increases with increasing time period. The reduction of the metal ions occurs fairly rapidly; more than 90% of reduction of zinc ions is complete within 4 Hrs. after addition of the metal ions to the plant extract. The metal particles were observed to be stable in solution even 4 weeks after their synthesis. By stability, we mean that there was no observable variation in the optical properties of the nanoparticle solutions with time[10].



Wavelength (nm)

Figure 2: UV-Vis spectrum of synthesized ZnO NPs

Scanning electron microscope (SEM) analysis was carried out by Electron Probe micro Analyzer JEOL MODEL No JXA8100.Thin film of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min. SEM provided further insight into the morphology and size details of the zinc oxide nanoparticles. Comparison of experimental results showed that the diameters of prepared nanoparticles in the solution have sizes between 1-100 nm of in case of 25 mints at 5000 rpm 25mints at 10000 &25mints at 30000rpm The size of the prepared nanoparticles was more than the size of nanoparticle which should be i.e. between 1-100 nm. The size was more than the desired size as a result of the proteins which were bound in the surface of the nanoparticles. The result showed that the particles were of spherical shape and sheet shape. The shape varies due to the concentration increased as showed in **Figure 3:**

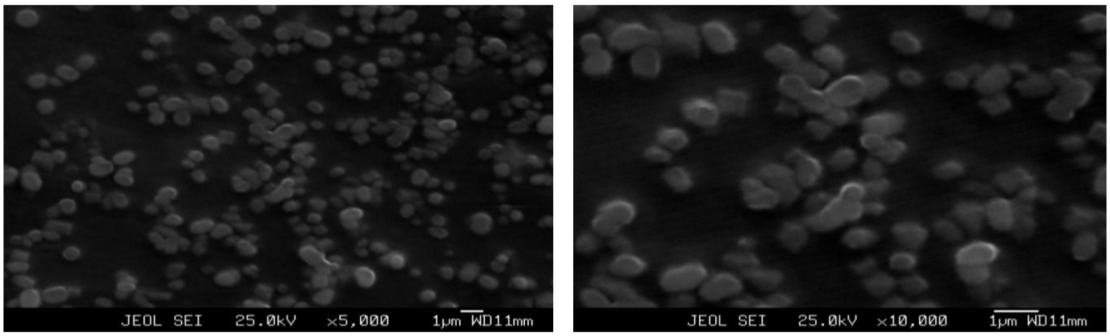


Figure 3: SEM analysis shows that size and shape of the ZnO nanoparticles synthesized by *Brassica oleraceae* at 1µm.

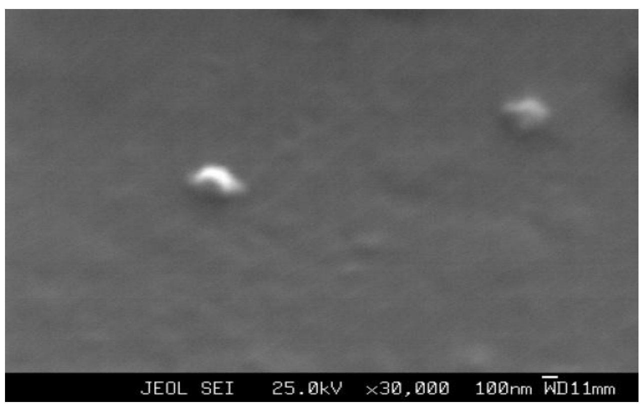


Figure 4: SEM analysis shows that size and shape of theZnO nanoparticles synthesized by *Brassica oleraceae* at 100nm.

Synthesized zinc oxide nanoparticles showed antibacterial activity against both Gram positive and negative bacteria **(Figure 5)**. The highest zone of inhibition was observed for 13mm in *E. coli, 14.5 mm in B..subtilis, 10 mm in C. botulinum, 15 mm in S. aureus, and 9..5 mm in V.cholrae* at 75µg/ml concentration. According to given results, 75µg/ml concentration of Ciplox showed maximum radius of zone of inhibition of 23mm against *E.coli*, 22mm against *B.subtilis*, 21mm against *C. botulinum* , 22.5mm against *S. aureus* and 21.5 against *V.cholrae*. The exact mechanism of the inhibition of the bacteria is still unknown, but some hypothetical mechanisms show that the inhibition is due to ionic binding of the zinc oxide nanoparticles on the surface of the bacteria which creates a great intensity of the proton motive force.

It was evaluated that the antibacterial activity of ZnO nanoparticles in Green Tea *(Camellia sinensis)* and it was reported the All Gram-negative bacteria had shown good sensitivity towards the green synthesized ZnO NPs for the concentration 20 µg mL^{-1}. It is quite interesting to note that all bacterial species tested in this study showed resistance to the synthetic antibiotic drug which in turn indicates the better antibacterial activity of the ZnO NPs than the commercially available synthetic drug. They investigated the antibacterial effect of ZnO nanoparticles and developed antibacterial agents a wide range of microorganisms to control the bacterial infection[11].

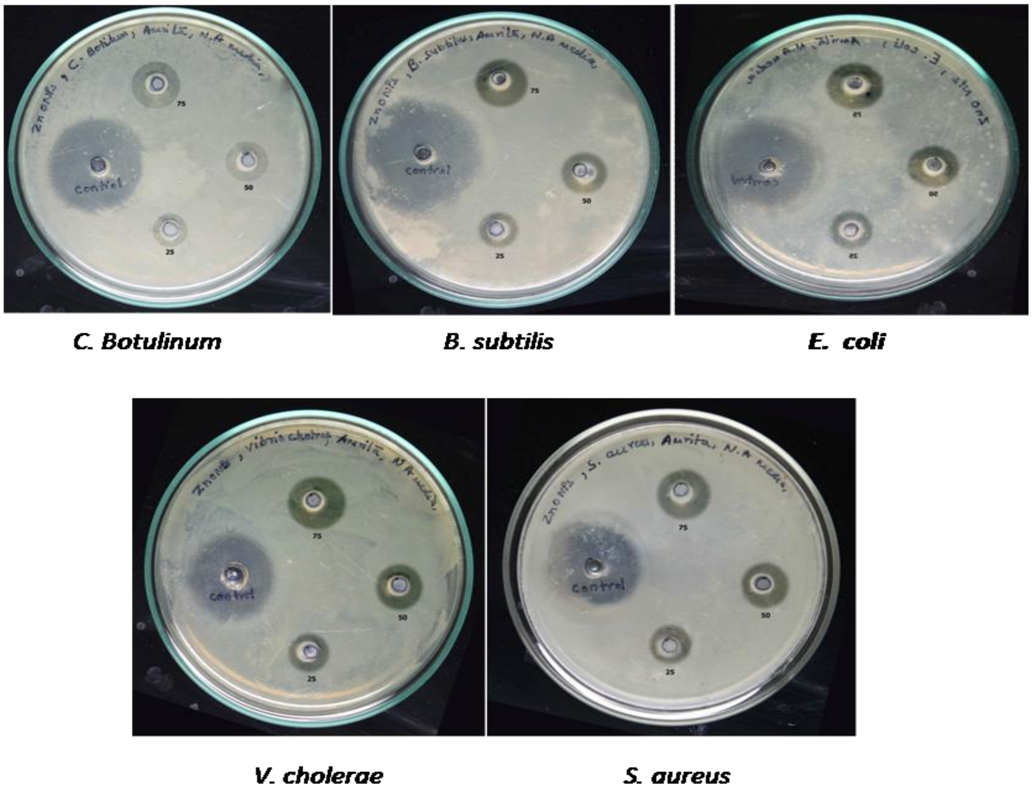
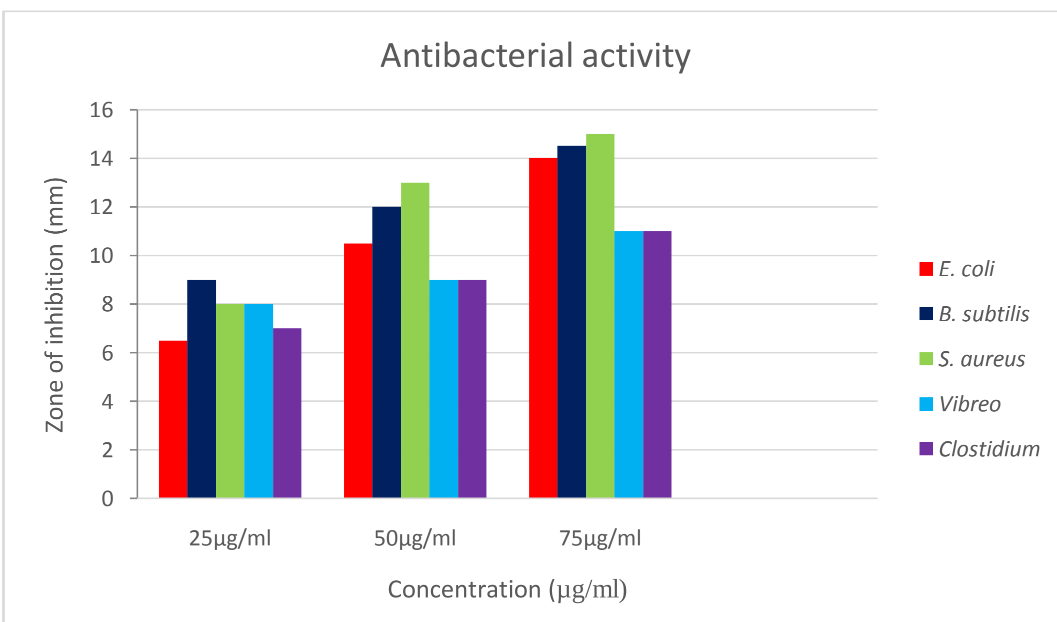


Figure 5: Antibacterial activity of ZnO nanoparticles against *a) Clostridium botulinum, b) Escherichia coli ,c) Bacillus subtilis, d) Staphylococcus aureus , e) Vibrio cholerae* at different concentration by agar well diffusion method

Graph 1:

Comparative Bar graph showing zone of inhibition introduced by different concentration of ZnO nanoparticles against *V. cholerae, B. subtilis, S. aureus, E.coli, C. botulinum.*



The minimum inhibitory concentration (MIC) was determined as the lowest concentration of zinc oxide nanoparticles that inhibited the visible growth of *E. coli, B. subtilis and S. aureus, V. cholare and C. botulinum* It was found that the MIC for *E. coli, B. subtilis and S. aureus*, was 50μg/ml and the lowest concentration of zinc oxide nanoparticles that inhibited the visible growth of *V. cholare and C. botulinum* was 25 μg/ml.

Table 1

MIC of ZnO nanoparticles against bacteria

Conc (ug /ml)	***MIC of ZnO nanoparticles* (ug / ml) against bacteria**				
	S. aureus	***B. subtilis***	***V. cholera***	***C. botilinum***	***E. coli***
100	-	-	-	-	-
50	*	*	-	-	*
25	+	+	*	*	+
12.5	+	+	+	+	+
6.25	+	+	+	+	+
MIC	50	50	25	25	50

(+) Growth (*) MIC and (-) growth

It was found that the MBC for zinc oxide nanoparticles was 25 µg/ml. These results indicated that the MBC of ZnO nanoparticles, the lowest concentration that retained its inhibitory effect resulting was the absence of growth (absence of turbidity) of a microorganism on nutrient agar media[12]. They reported the approximately similar result of MIC and MBC, ZnO nanoparticles are effective antibacterial agents both on Gram-positive and Gram-negative bacteria[7]. In this study, results of MIC and MBC show that the MIC and MBC were 0.1 and 0.8 µg/ml, respectively. The ratio of MBC/MIC was 8 ug/ml. These results indicate that zinc oxide nanopaticles had antibacterial effects on *all* bacteria. These results are consistent with the results of MIC and MBC. The antibacterial activities increased as the concentration of zinc oxide nanoparticles increased.

CONCLUSION:

The green synthesized zinc oxide nanoparticles were great interest due to their eco- friendliness, nontoxic, economic prospects, and feasibility and short time for synthesis may be wide range of application in nanomedicine, catalysis medicine mainly for the pharmaceutical industry for development of new formulations against the bacterial strains which are developing resistance to traditional antibiotics.

Zinc oxide nanoparticles were successfully produced by the *Brassica oleraceae* leaves extract assisted synthesis. The use of plant extracts avoids the usage of harmful and toxic reducing and stabilizing agents. Zinc nanoparticles can exist in ions only in the presence of strong oxidizing substances. The environmental conditions will affect the stability of nanoparticles.

The antibacterial activity increased with increase in surface – to – volume ratio due to a decrease in particles size of nanoparticles. Here, ZnO nanoparticles showed excellent bactericidal potential. Our results indicate that nanomaterials were most effective against Gram- positive and Gram – negative bacterial strains.

The rapid biological synthesis of zinc oxide nanoparticles using *Brassica oleraceae* leaves extract provides environmental friendly, simple and efficient route for synthesis of benign nanoparticles. The synthesized nanoparticles were of spherical and sheet shaped and the estimated sizes were 1-100 nm. The size were as the nanoparticles were surrounded by a thin layer of proteins and metabolites such as terpenoids having functional groups of amines, alcohols, ketones, aldehydes, etc., which were found from the characterization using UV-vis spectrophotometer, SEM, techniques. All these techniques it was proved that the concentration of plant extract to metal ion ratio plays an important role in the shape determination of the nanoparticles. The higher concentrated nanoparticles had sheet shaped appearance where as the lower concentrations showed spherical shaped. The sizes of the nanoparticles in different concentration were also different which depend on the reduction of metal ions. From the technological point of view these obtained by zinc oxide nanoparticles have potential applications in the biomedical field and this simple procedure has several advantages such as cost-effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production These nanoparticles showed abroad spectrum antimicrobial activity against both Gram positive and Gram negative bacteria. Investigation on the antibacterial activity of synthesized zinc oxide nanoparticles using *brasica* extract against *S.aureus, B.subtilis, V.cholrae, C. botulinum, and E. coli*. It has high potential as antimicrobial agent in pharmaceutical, food, and cosmetic industries.

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