Studies on antibacterial activity of *Aegle Marmelos* mediated Y_2O_3 nanoparticles

[1]Sanjay R. Kale*, [2]Varsha S. Garule, [2]Komal R. Jagtap and [3]Dilip R. Thube.

[1]Department of Chemistry, Tuljaram Chaturchand College of Arts, Science and Commerce, Baramati, 413102, Dist. Pune Maharashtra (INDIA),
[2]Department of Microbiology, Tuljaram Chaturchand College of Arts, Science and Commerce, Baramati, 413102, Dist. Pune Maharashtra (INDIA),
[3]Professor, Department of Chemistry and Research Center, New Arts Commerce and Science College, Parner,414302, Dist-Ahmednagar. Maharashtra (INDIA)
E-mail: *kalesanjay29@gmail.com* (* Corresponding Author)

Abstract: Green synthesis of Yttrium oxide (Y_2O_3) nanoparticles was carried out using *Aegle Marmelos* (Bael) leaves aqueous extract as a reducing and capping agent. The synthesized metal complex was calcined at 800ºC.The produced nanoparticles (NPs) were characterized by using various instrumental techniques. The characteristic study clearly indicates that the synthesized Y_2O_3 nanoparticles are a crystalline material having cubic structure of agglomerated Y_2O_3 NPs with particle size ~ 34nm identifies the presence of elements yttrium and oxygen with Y–O–Y and O–Y–O metal oxide linkages. This report is on the results of the antibacterial studies carried out by using synthesized *Aegle Marmelos* extract mediated Y_2O_3 NPs revealed an increasing rate of antibacterial behavior with selected microorganisms.

1. Introduction:

Nanotechnology is one of the fastest developing sciences during last few years. Nanosized inner transition metal oxides, in variety of morphologies have attracted a great deal of attention due to their superior physico-chemical properties which are associated with various potential applications in the fields like catalysis [1], delivery vehicles for drugs [2,3], antimicrobial activity [4,5].

Nowadays green route can be adopted in the synthesis of metal and metal oxide nanoparticles using plant material. The plant extract mediated method for the synthesis of nanoparticles is one of the easy, safer and non-toxic methods which have gained more interest towards environmentally conscious products. The metal and metal oxides nanoparticles have been considered as promising material that possesses remarkable antibacterial properties caused by their high surface area[6].The bacteria must be controlled because it causes infection to human, contributes to several non-infectious chronic diseases and also contaminates in the open water. To control and minimize the problems caused by the bacteria, Y_2O_3 NPs is one of the key material which can leads to cell death of bacteria in a non-toxic way to environment. Therefore in this study we use leaves extract of *Aegle Marmelos* plant to synthesize Y_2O_3 NPs and further applied for antibacterial activity. The *Aegle Marmelos* is a traditional plant also known as Beal belongs to Rutaceae family and have been used in medicine and medical applications to cure ophthalmia, catarrh, deafness, aggravations, diabetes and asthma throughout centuries in India. The leaves extract of *Aegle Marmelos* was used to synthesize ZnO NPs[7] and Silver NPs[8] for their antimicrobial activity had been reported. Biosynthesis of Y_2O_3 NPs using *Acalypha indica* leaf extract and their antibacterial study[9] has been reported. Green synthesis and characterization of Y_2O_3, CuO and $BaCO_3$ NPs using *Azadirachta Indica* (Neem tree) fruit aqueous extract has been reported[10].Y_2O_3 is a well known and widely used as a host material in the field of biological imaging and photodynamic therapy[11], still there is no report found on antibacterial activity of *Aegle Marmelos* extract mediated Y_2O_3 NPs.

The work presented in this report includes studies on antibacterial activity of *Aegle Marmelos* mediated Y_2O_3 nanoparticles with selected human pathogen.

2. Materials and Method to study the Antibacterial Activity

Green synthesis of *Aegle Marmelos* mediated Y_2O_3 NPs was carried out by using Yttrium nitrate hexahydrate [$Y(NO_3)_3$ $6H_2O$] was purchased from Alfa Aesar and aqueous leaves extract of *Aegle Marmelos* (Bael tree) by homogenous precipitation method. The precipitate formed is dried and calcined in a muffle furnace at 800^0C for 4 hour to obtain Y_2O_3 NPs.The *Aegle Marmelos* mediated Y_2O_3 NPs was applied for antibacterial activity against *Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Serratia marcescens (S. marcescens) and Klebsiella pneumonia (K. pneumonia)*.The studies on the antibacterial activity was done using disc diffusion method and Turbidimetric method by using colloidal solution of Y_2O_3 NPs.

3. Results and Discussion

Recently nanomaterials have become a tool against multidrug- resistant bacteria[12, 13]. These nanomaterials can be used as nano drugs that can act individually or along with antibacterial compounds against bacteria. Nanomaterials are also used as drug delivery systems which gave more therapeutic efficacy and enhanced physicochemical properties. The metal oxide NPs are one of the most studied nanomaterials against multidrug resistant bacteria. The antibacterial properties associated with metal oxide NPs are due to their special characteristics like increased surface to volume ratio with decrease in the particle size, nanoparticles stability, Van der Waals forces (hydrophobic interactions) and electrostatic attraction. These characteristics allow NPs to show their antibacterial activity through multiple mode of mechanisms which includes damage to the membrane and bacterial cell wall, damage to proteins and internal components of bacteria, release of ions, DNA damage and oxidative stress with generation of reactive oxygen species[14].

As mentioned above, the smaller the size of NPs, the greater their surface to volume ratio. The increase in surface to volume ratio of NPs improves their ability to interact with various components of bacterial cell and exercise their antibacterial activity[15]. The size and shape of NPs is one of the main factors of their antibacterial power along with other factors like type of synthesis, precursors and parameters used. It was reported that the NPs with smaller size and spherical shape had higher antibacterial activity due to quick response and release of metal ion that can penetrate the defense of bacteria[16].

Another factor reported for antibacterial effect was the electric charge present on the surface of metal oxide NPs[17]. There are three types of microorganisms- Gram positive, Gram negative and endospores. The NPs showed antibacterial activity against Gram positive and Gram negative bacteria while endospores were least sensitive to metal oxide NPs[18]. The bacteria are able to regulate the electrical charge on their surfaces and therefore they can repel NPs with positive, negative or neutral charges from their surfaces[19]. The metal oxide NPs with positive charge are found to be most effective against Gram positive and Gram negative bacteria. This is due to attraction occurs between NPs and bacterial surface barring negative charge. In some report[20] it was suggested that there are some bacterial species which have mechanisms to generate resistance to the charge present on the surface of NPs. This may be due to modulating the electrical charge on their surface by envelope stress response (ESR) and protect the integrity of bacterial cell.

There was a report[21] which suggest that the metal oxide NPs releases the toxic ions into bacterial cell causing additive, antagonistic and synergistic effects resulting in the death of bacterial cell. Also there were some reported mechanisms[22] for antibacterial activity like generation of reactive oxygen species (ROS) and oxidative stress by metal oxide NPs which causes damage to the internal components of bacterial cell such as structural proteins, enzymes, DNA, respiratory chain[23].

The antibacterial activity of Y_2O_3 nanoparticles (NPs) was determined against representative Gram-positive microorganisms i.e. *Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens and Klebsiella pneumonia*. The *E. coli* is the characterized bacterium mostly used as model bacterial systems for different antibacterial testing protocols, The *P. aeruginosa* is involved in hospital acquired infections (HAIs), urinary

tract infections and wound infections, The *S. marcescens* is found in soil, water, skin flora and manmade environments which cause diseases in human and animals, while *K. pneumonia* is an opportunistic gut bacteria normally live in intestines and feces, which are harmless when they are in intestine but if they spread to another part of body they can cause severe infections in the urinary and respiratory tracts.

The antibacterial activity of Y_2O_3 nanoparticles was examined by disc diffusion method. In this method the Y_2O_3NPs laden disk is prepared by keeping the disks in 5 ml colloidal solution of Y_2O_3NPs for two days. These disks absorb Y_2O_3NPs and become dry free form chloroform. For the antibacterial susceptibility testing by the Kirby- Bauer method, the cultures of different microorganisms under study were used as reference strains. The bacterial suspensions were applied uniformly on the surface of Muller Hinton agar (MHA) plate in the concentration range of $10^5 – 10^6$ CFU ml^{-1} before placing the Y_2O_3NPs laden disk. The strains were cultured on nutrient agar plate. The plates with the disk were incubated aerobically at 35^0C for one day. Afterwards the average diameter of the inhibition zone surrounding the disk was measured with scale. Fig. 1- 4 shows plates with *E. coli, P. aeruginosa, S. marcescens* and *K. pneumonia* bacterial suspension were applied with NPs laden disk and antibiotic impregnated disks. The diameter of inhibition zone surrounding the disk in presence of Y_2O_3NPs in *E. coli, P. aeruginosa* and *S. marcescens* bacterial suspension are 8, 9.5 and 11 mm, respectively while *K. pneumonia* do not show any remarkable zone of inhibition. (Table 1)

Fig.1: Inhibition of *Escherichia coli*

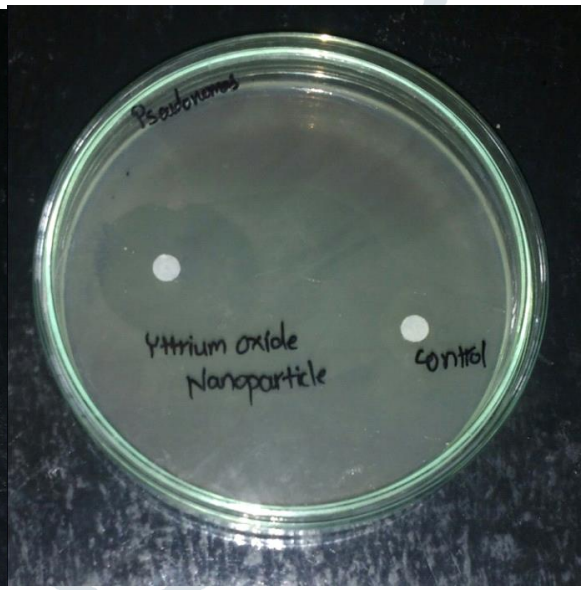

Fig.2: Inhibition of *Pseudomonas aeruginosa*

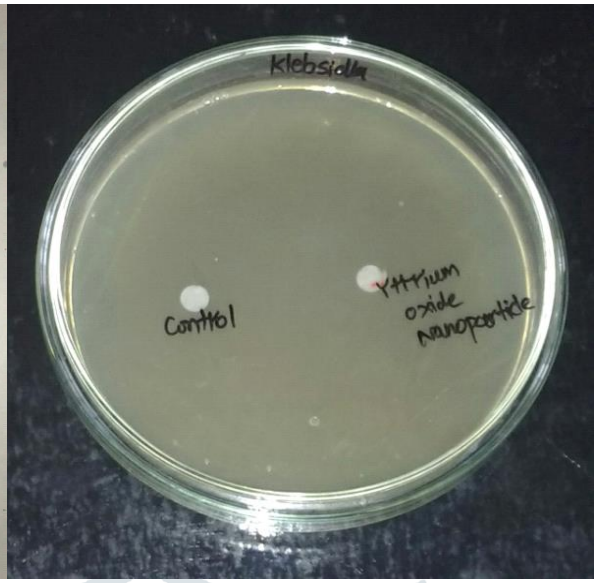

Fig.3: Inhibition of *Serratia marcescens* Fig.4: Inhibition of *Klebsiella pneumonia.*

Table 1: Inhibition in growth of microorganisms due to Y_2O_3 nanoparticles.

Sr. No.	Indicator Microorganism taken for study	Inhibition zone diameter (mm)
1	*Escherichia coli*	8
2	*Pseudomonas aeruginosa*	9.5
3	*Serratia marcescens*	11
4	*Klebsiella pneumonia.*	--

The antibacterial effects of Y_2O_3 nanoparticles are also studied by determining the minimum concentration required to inhibit the growth of microorganisms under study. In this method, the antibacterial behavior of Y_2O_3 NPs against E. *coli, P. aeruginosa, S. marcescens* and *K. pneumonia* in Luria Bertani (LB) broth is studied. For that purpose, 24 hour old cultures were inoculated into LB broth supplemented with various concentrations of Y_2O_3 nanoparticles (2, 4, 6, 8 and 10 $\mu g.ml^{-1}$) while Y_2O_3- free LB broth was used as control. The broth containing tubes were incubated at room temperature under stirring for 24 hour and the vulnerability of the tested microorganisms was observed by determining optical density (O.D.) values at 600 nm using UV-Visible spectrophotometer. It reflects the growth rate of different microorganisms, E. *coli, P. aeruginosa, S. marcescens* sand *K. pneumonia* with various concentrations of Y_2O_3 nanoparticles. The growth curves (Fig. 5 - 8) represents the inhibitory effect of various concentrations of Y_2O_3 nanoparticles. This study shows that the growth rate of microorganisms was decreased with increase in concentration of Y_2O_3 nanoparticles and the maximum inhibition for growth was obtained at $10\mu g.ml^{-1}$ (Table 2)

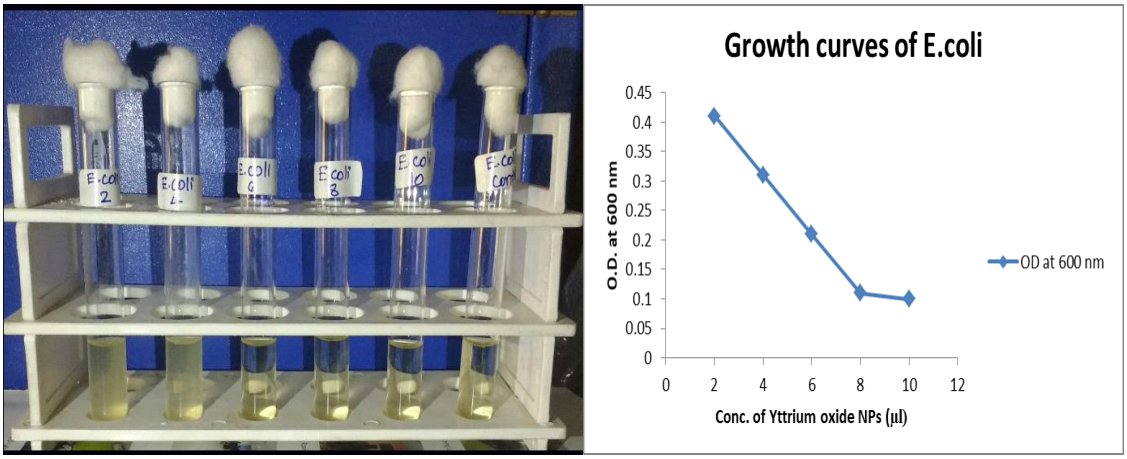

Fig.5: MIC of *Escherichia coli*

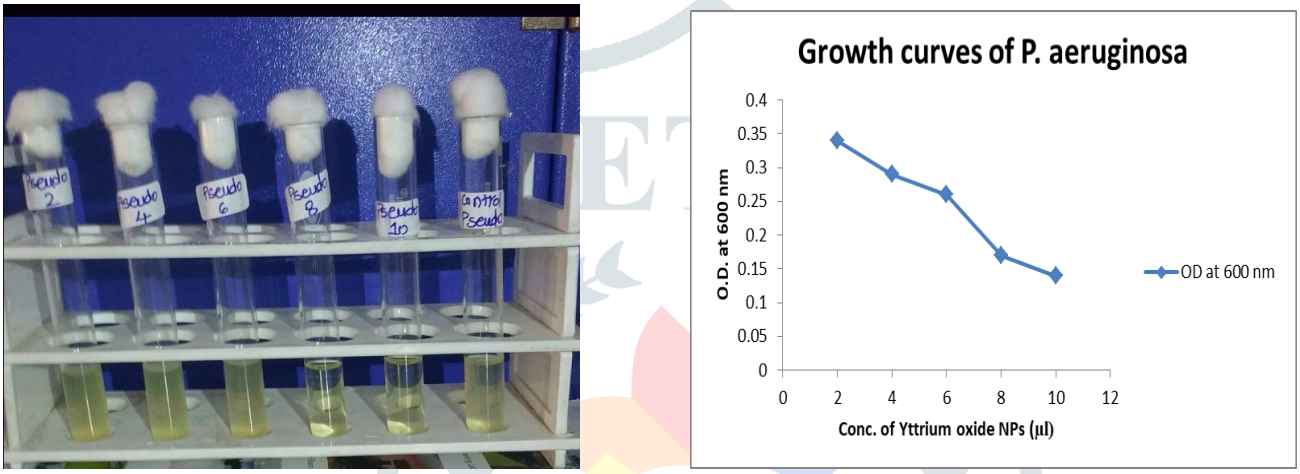

Fig.6: MIC of *Pseudomonas aeruginosa*

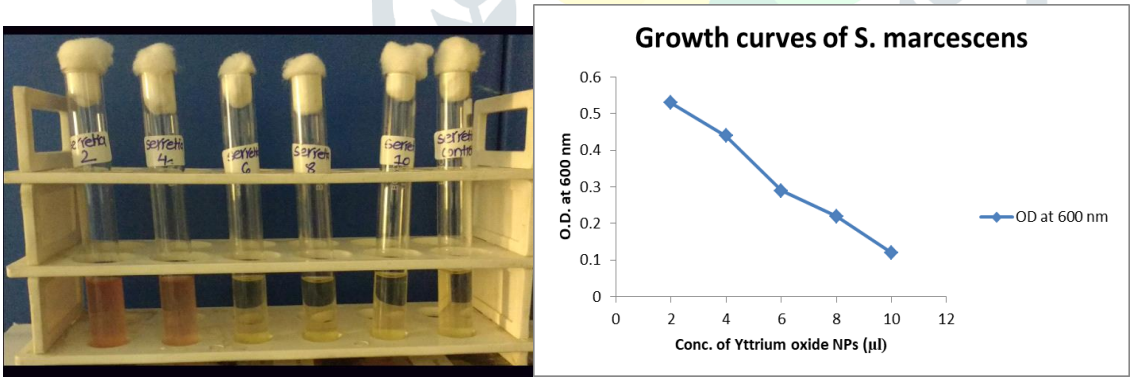

Fig.7: MIC of *Serratia marcescens*

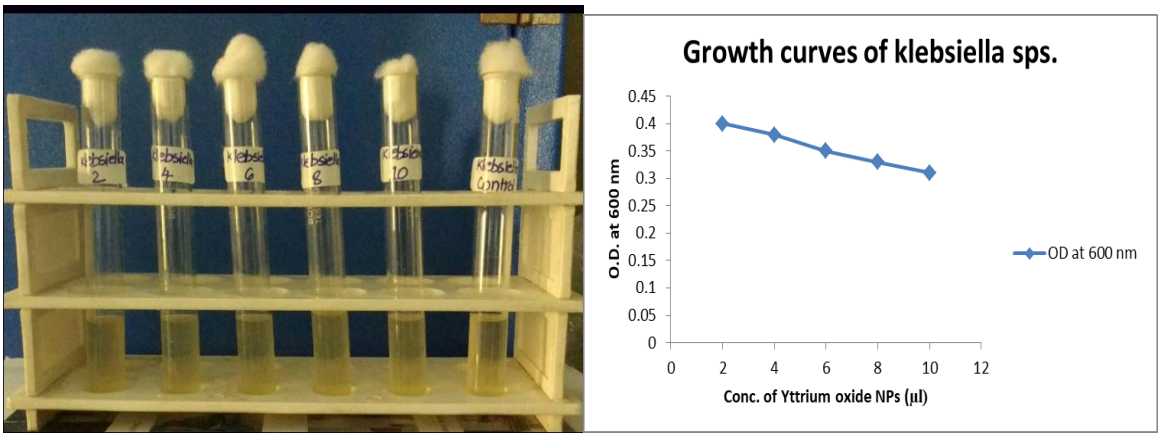

Fig.8: MIC of *Klebsiella pneumonia*

Table 2: MIC in µg.ml^{-1} of Y_2O_3 nanoparticles against tasted microorganisms.

Microorganism →	E. *coli*	*P. aeruginosa*	*S. marcescens*	*K. pneumonia*
MIC in µg.ml^{-1} of Y_2O_3 nanoparticles→	8	8	10	10

The detailed mechanism of antibacterial activity and inhibition of growth of microorganisms using Y_2O_3 nanoparticles was not very well known yet, but it was believed that in presence of Y_2O_3 NPs the cellular proteins in the microorganisms became inactive when they penetrates into microorganisms and inactivated their enzymes. During this process H_2O_2 gas liberates which is responsible for the death of cells of microorganisms. The heavy metal like Yttrium is toxic which react and bind with cellular protein molecule, inhibits the cellular mechanism and causes death of microorganism.

This experimental study confirmed that the Y_2O_3 nanoparticles can show antibacterial behavior and therefore used as effective growth inhibitors against various microorganisms during preparation of various antibacterial control systems and effective inhibitory nanomedicines.

4. Conclusion

In the field of nanotechnology a considerable attention has been directed towards use of plant materials to synthesize metallic oxide nanoparticles with green approach by developing suitable, reliable and eco-friendly method. The present study fulfils the objectives of green synthesis by adopting a simple, fast and economical approach for preparation of *Aegle Marmelos* mediated Y_2O_3 nanoparticles. The experimental results confirmed that the synthesized *Aegle Marmelos* mediated Y_2O_3 nanoparticles are stable with an average size about 34.58 nm. It was proved that these nanoparticles have antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens and Klebsiella pneumonia* bacteria.

The achievement of such green synthesis of Y_2O_3 nanoparticles could be the alternative and be useful in the field of biomedicine for their antibacterial properties. Therefore, this green method is one of the eco-friendly, economical and effective process to synthesis Y_2O_3 NPs and it may lead to the further study on use of *Aegle Marmelos* in the area of biomedical and nanotechnology.

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