



## **A new cost effective potassium based LaFeO<sub>3</sub> perovskite for antimicrobial application**

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**Abstract :** The present work is aimed at investigating the antimicrobial activity of Lanthanum Potassium Ferrate (LKFO) nanoparticles against *P. aeruginosa*- a gram-negative bacteria and *S. aureus* - a gram-positive bacteria. For comparative study, series of LKFO nanoparticles was prepared and assessed for antibacterial efficacy. The average size of the synthesized nanoparticles varied from 20 nm to 100nm and X-ray diffraction pattern showed the formation of a single phase LKFO of an perovskite crystal structure after annealing the precursor at 800<sup>o</sup>C for 8 hr. The antibacterial activity of synthesised nanoparticles were tested on gram positive and gram negative bacteria according to the radial diffusion assay (RDA) for antibacterial agents. The results indicate promising antibacterial activity of La<sub>x</sub>K<sub>1-x</sub>FeO<sub>3</sub> (x= 0.1, 0.2, 0.3) on both gram-positive and gram-negative bacteria. The presence of potassium in the perovskite catalyst offers substantial benefits for surface mobility and electron donor properties. MIC values for *S.aureus* and *P.aeruginosa*, were in the range of 50 - 400 mg/mL, the enhanced antibacterial activity is attributed to increased doping of potassium (K) stoichiometric ratio of the perovskite nanoparticles. MBC studies showed that the growth of *S.aureus* and *P.aeruginosa* could be ceased above 100 mg/mL of La<sub>x</sub>K<sub>1-x</sub>FeO<sub>3</sub> (x= 0.1, 0.2, 0.3). Thus the data supports the use of such nanoparticles as a potential antimicrobial agents specially with regards to water purification technology.

**Keywords :** Potassium based LaFeO<sub>3</sub> perovskite, antibacterial application, *S.Aureus*, *P. Aeruginosa*.

### **Introduction and Experimental**

Water covers almost two-thirds of Earth's surface. Still, lack of clean water has been a worldwide problem to humanity for many years. Nature has its own mechanism to recycle water and provide enough quantity of clean water to us. However, uncontrolled human population growth and unplanned industrialization have disrupted the natural purification processes, leading to a shortage of potable water [1]. Nearly 90% of all diseases in most of the developing countries are caused due to the consumption of impure water. A major portion of the drinking water sources worldwide are found to be contaminated with various toxins and

pathogenic microbes, mostly due to the release of untreated man-made wastes or wastewater to these sources. Therefore, the proper treatment of wastewater prior to its release is very important for protecting our ecosystem. In order to address these issue, it is the challenge for research, development, and technology institutions to come up with cost-effective alternative microbial wastewater treatment technologies.

A great deal of research using perovskite catalyst has been carried out for auto-exhaust treatment for oxidation reaction. Some applications also have been reported on water and waste water treatment, however, their application in the field of bacterial disinfection for drinking water and sewage waste water treatment has very been rarely reported [2, 3]. Perovskite compound can tolerate significant partial substitution and non-stoichiometry, while still maintaining the perovskite structure. The performance of a supported metal or alloy catalyst can be directly related to the size and spatial distribution, as well as, the composition of the catalyst[4].

Antibacterial agents such as Ag Nanoparticles (NPs) are very important in the textile industry [5, 6, 7], water disinfection [8, 9, 10], medicine[11], and food packaging[12]. It has been reported that Ag nanoparticles are active biocides against gram-positive and gram negative bacteria including *E. coli*, *S. aureus*, *K. pneumoniae* and *P. aeruginosa* [13, 14].

The interest in inorganic disinfectants such as metal oxide nanoparticles (ZnO, TiO<sub>2</sub>, CuO NPs etc.) is increasing [15, 16, 17]. Occurrence of NP breakthrough into finished water during drinking water treatment, after coagulation/flocculation/sedimentation, as well as membrane filtration has also been reported[18].

Therefore, it is very important to select some innovative alternative for such application. In this study, potassium based perovskite nanocatalyst (La<sub>x</sub>K<sub>1-x</sub>FeO<sub>3</sub> {x= 0.1, 0.2, 0.3}) has been selected and studied against *P. aeruginosa*- a gram-negative bacteria and *S. aureus* - a gram-positive bacteria as such composition of perovskite catalyst has not been studied earlier. The possible uniqueness of its formulation, synthesizing brings in the right properties for antibacterial application. The presence of potassium in the perovskite catalyst offers substantial benefits for surface mobility [19, 20] and electron donor properties [21].

Lanthanum based perovskite oxides have been the most frequently studied and have demonstrated remarkable performance in different types of catalysis [22, 23]. Substituting B-cations with a reducible early transition metals such as Co, Mn, Fe provide redox active sites that facilitates catalytic reaction. Synergistic effect, which is due to combination of two different ions at B-site, leads to enhanced catalytic activity [24]. As the same time, it was also observed that the addition of alkali metals to the catalyst formulation offers substantial benefit for surface mobility [19, 20] and electron donor properties [21]. Potassium is a well known catalyst for carbon gasification and, consequently, it was used as catalysts for soot combustion [25, 26, 27, 28]. In our previous work LaCoO<sub>3</sub> doped Fe have found to be effective for antibacterial application and the probable mechanism of antimicrobial activity by perovskite material is also discussed [29]. LaCo<sub>x</sub>Fe<sub>1-x</sub>O<sub>3</sub> also have been studied for methane oxidation catalysis [30, 31]. Lanthanum doped NaTaO<sub>3</sub> and MTiO<sub>3</sub> (M = Sr, Ba, Mn, Co, Fe) have been studied for photocatalytic applications [32-33]. However, application of La based perovskites in the field of bacterial disinfection is limited. LaCo<sub>x</sub>Fe<sub>1-x</sub>O<sub>3</sub>, La<sub>0.67</sub>Ca<sub>0.33</sub>MnO<sub>3</sub>/ (La<sub>0.5</sub>Eu<sub>0.5</sub>)<sub>0.67</sub>Ca<sub>0.33</sub>MnO<sub>3</sub>, CaTiO<sub>3</sub>, Ba(Zr<sub>x</sub>Ti<sub>1-x</sub>)O<sub>3</sub>, SrTi<sub>1-x</sub>Fe<sub>x</sub>O<sub>3-1</sub>, also have been used as an anti-microbial agent [29,34, 35, 36, 37].

Perovskite metal oxide has been synthesized via various approaches including high temperature solid state reaction [38], co-precipitation method [39], liquid mixed technique [40], wet chemical routine [41], citrate sol-gel method [29] and a few other methods. The inexpensive citrate sol-gel method has a better control over the chemical composition of products to form a catalyst having good surface area and porosity. Hence, this method was adopted for synthesis of La<sub>x</sub>K<sub>1-x</sub>FeO<sub>3</sub> with x = 0.1,0.2,0.3 perovskite, which required low temperature (800<sup>o</sup> C) for calcinations to obtain the desired single phase powder. The feasibility of utilizing this material for antibacterial applications was evaluated and reported for the first time.

### Synthesis of the La<sub>x</sub>K<sub>1-x</sub>FeO<sub>3</sub> (LKFO) Nanoparticles

A series of La<sub>x</sub>K<sub>1-x</sub>FeO<sub>3</sub> with x = 0.1,0.2,0.3 were synthesised by citrate precursor sol-gel method. The salt precursors used for the synthesis were: La(NO<sub>3</sub>)<sub>3</sub>.6H<sub>2</sub>O (Loba Chemie, 99% AR), Fe(NO<sub>3</sub>)<sub>3</sub>.9H<sub>2</sub>O (Loba Chemie, 98% AR), KNO<sub>3</sub> (Loba Chemie, 98% AR) and Citric acid (M: Citric acid, 1:1) were dissolved in distilled water, the solutions were stirred with a magnetic stirrer at the temperature of 100°C. Stirring was continued till the formation of gel for approximately 2 h. The gel was kept in a preheated furnace at

200 °C to give the formation of amorphous precursor, which was further annealed at 800°C for 8 hour to obtain the respective crystalline perovskite nanoparticles.

## Characterisation Techniques

### X-Ray Diffractometer

The Crystal structure of LKFO perovskite nanoparticles were analysed in terms of X-Ray Diffraction (XRD) by using a X-Pert PRO X-Ray Diffractometer, at room temperature (29 °C) in the range (10-80 degree) with Cu-K  $\alpha$  radiation of wavelength  $\lambda = 0.1540598$  nm.

### Scanning Electron Microscopy

To Characterize mean particle size and morphology of LKFO perovskite nanoparticles, SEM (Scanning electron microscope) was performed using a 200 kV JEOL (Tokyo, Japan)

### BET Surface Area Measurement

Heterogeneous catalysis is a surface phenomena, and the availability of large active surface for a given mass of catalyst is crucial for effective catalysis. Surface area is measured using the BET (Brunauer, Emmet, and Teller) technique, based on the physical adsorption of an inert gas at a constant temperature [42], by using Smartsorb-93 BET surface area analyser.

### Maintenance of bacterial cultures

The 2 pathogenic bacterial cultures used for the antimicrobial assay were *Pseudomonas aeruginosa* MTCC 1688 (gram-negative) and *Staphylococcus aureus* MTCC 96. The cultures obtained from Guru Nanak Institute of Research and Development (GNIRD) of G. N. Khalsa College, Matunga were maintained on nutrient agar medium at 4 °C till further use.

### Inoculum preparation

A single colony was inoculated in 50 ml of sterile Mueller Hinton broth in 250 ml conical flask and incubated at 37 °C/150 rpm for 24 h. The absorbance of bacterial cells was adjusted to 0.1 at 620nm corresponding to  $1.0 \times 10^8$  cfu/ml for the antibacterial assay [43].

### Antibacterial assay

Preliminary assessment of antibacterial activity of the nanomaterials was primarily done using agar well diffusion technique and subsequent quantification was carried by Minimum Inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) against the 2 selected pathogenic bacteria.

### Agar well diffusion method

The agar well diffusion method was carried out as per the guidelines given by CLSI [44]. One ml of the inoculum was mixed thoroughly in 20 ml of Mueller Hinton agar and poured into flat base Petri plates with 9 cm diameter. Post agar solidification, wells were bored using 8 mm of cork borer. Fifty  $\mu$ l of the nanomaterials were transferred to respective wells and the plates were left standing for 15 minutes at 4°C to let the nanomaterials get diffused prior to incubating the plates at 37 °C for 24 h. Post incubation the zone of inhibition was measured in mm. Negative control was maintained using Dimethyl Sulphoxide (DMSO). Ciprofloxacin (10 ppm) was used as positive control [43]. All experiments were carried out in triplicates and the values obtained for diameter of zone of inhibition were expressed as mean $\pm$ SD.

### Determination of MIC and MBC

Minimum Inhibitory Concentration of perovskite nanoparticles is the lowest concentration of the nanomaterial that inhibits the visible growth of a microorganism after an overnight incubation. MBC was determined by 2 fold broth-dilution method. In the present experiment a stock solution of Mueller Hinton medium with Perovskite nanomaterials was prepared and further diluted serially using Mueller Hinton broth in

96 well microtiter plate to obtain a range of 50-400  $\mu\text{g/mL}$ . Each set was inoculated aseptically with 50  $\mu\text{L}$  of respective bacterial suspension ( $10^6$  CFU/mL) of the 2 selected bacterial cultures. Positive control was maintained by inoculating culture in growth medium without test samples but with standard antibiotic (Ciprofloxacin). The negative control well contained uninoculated medium and DMSO. The plates were incubated at  $37^\circ\text{C}$  for 24 h. The MIC of perovskite nanoparticles was determined post the incubation period by adding 40  $\mu\text{l}$  p-iodonitrotetrazolium salt (INT dye) and incubating at ambient temperature for 30 min. Viable bacteria reduced the yellow coloured dye to pink. The MIC of each sample was determined based on the lowest concentration of the nanoparticles that prevented this conversion and resulted in complete inhibition of bacterial growth [13, 34]. Minimum Bactericidal Concentration (MBC) was evaluated by streaking a loopfull of the sample from the wells with concentrations corresponding to MIC and above it on fresh Mueller Hinton agar plates. The MBC was determined as the least concentration of the nanomaterial sample which led to no bacterial growth indicating 99.5% killing of the original inoculums [36].

## Results and Discussion

### Characterisation of the Catalyst

#### X-Ray Diffractions of LKFO Nanoparticles

The typical XRD patterns of the  $[\text{La}_x\text{K}_{1-x}\text{FeO}_3]$  ( $x = 0.1, 0.2, 0.3$ ) nanoparticles annealed at  $800^\circ\text{C}$  for 8 h are shown in Figure 1. The peak positions ( $2\theta$ ) of samples as represented in the figure, exhibit the single phase perovskite cubic crystal structure.

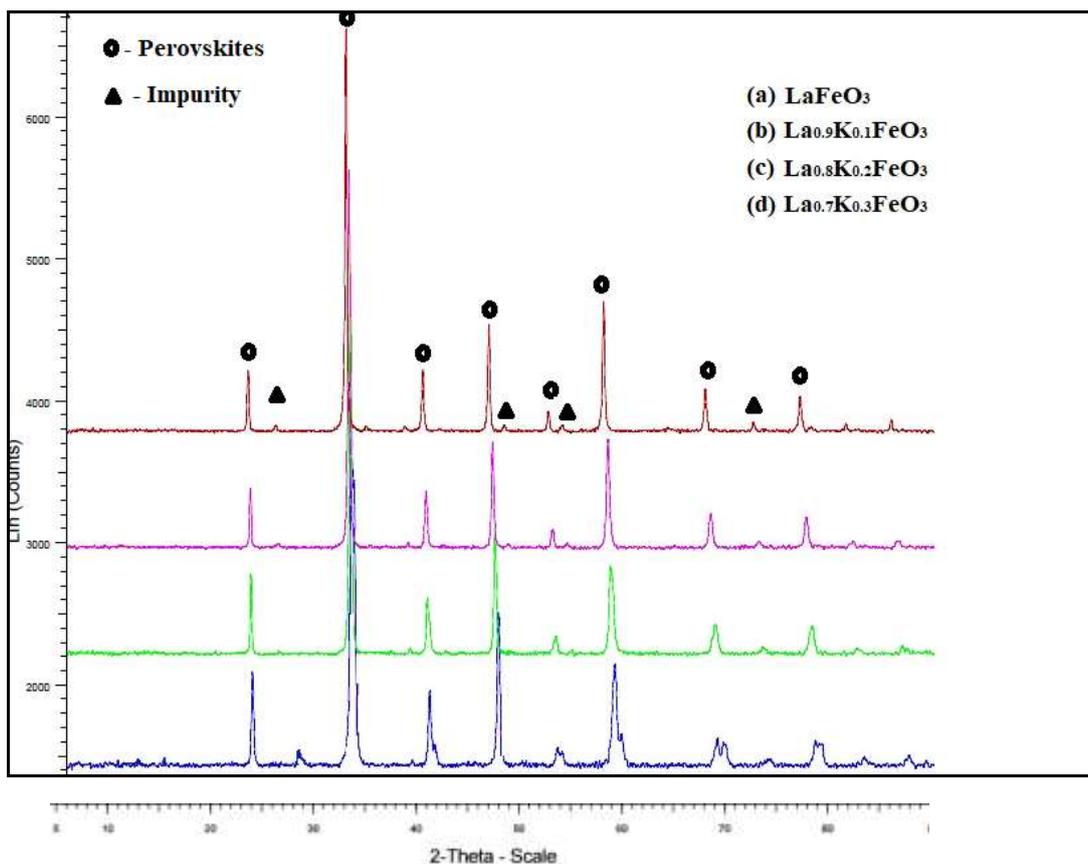


Figure 1. X-Ray Diffractions of (a)  $\text{LaFeO}_3$  (b)  $\text{La}_{0.9}\text{K}_{0.1}\text{FeO}_3$  (c)  $\text{La}_{0.8}\text{K}_{0.2}\text{FeO}_3$  and (d)  $\text{La}_{0.7}\text{K}_{0.3}\text{FeO}_3$

### Scanning Electron Microscopy images of LKFO series nanoparticles

The most fine, highly dispersed phase can be seen in Figure 2(c) and Figure 2(d) with A site potassium substitution. These results provide useful insight into the grain size structure and morphology. SEM results showed that perovskite nanoparticles formed in the range of particle size between 20 nm to 100 nm.

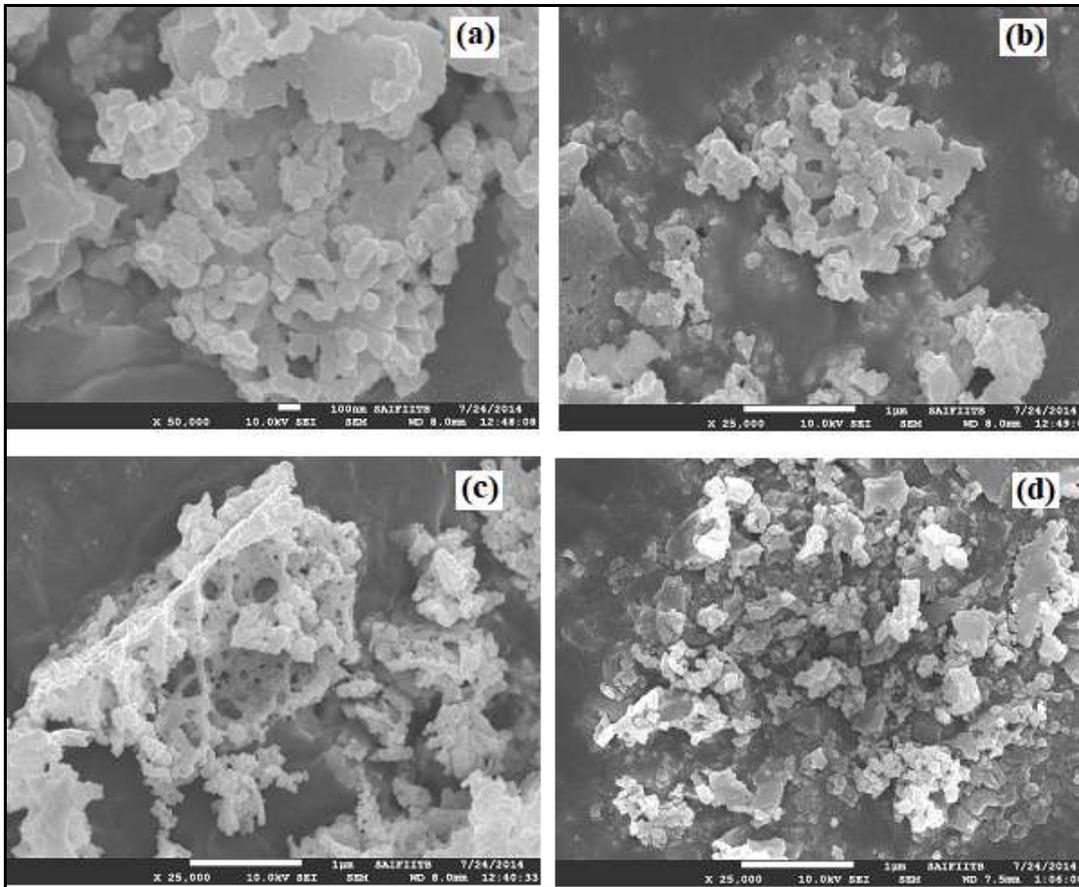


Figure 2. SEM micrograph of (a)  $\text{LaFeO}_3$  (b)  $\text{La}_{0.9}\text{K}_{0.1}\text{FeO}_3$  (c)  $\text{La}_{0.8}\text{K}_{0.2}\text{FeO}_3$  and (d)  $\text{La}_{0.7}\text{K}_{0.3}\text{FeO}_3$

### BET Surface Area Measurement

The calculated surface areas are summarized in Table 1.  $\text{LaFeO}_3$  has specific surface area of about  $8.82 \text{ m}^2/\text{g}$ . The grain size of K doped  $\text{LaFeO}_3$  are smaller than those of pure  $\text{LaFeO}_3$ . It is noteworthy that the BET surface area of K doped materials are larger than pure  $\text{LaFeO}_3$  and as the K stoichiometry ratio increase in A-site of  $\text{LaFeO}_3$ , the specific surface area increases.  $\text{La}_{0.7}\text{K}_{0.3}\text{FeO}_3$  has a higher surface area of about  $14.96 \text{ m}^2/\text{g}$ , which can be attributed to increasing stoichiometry ratio of K in A site of  $\text{LaFeO}_3$ . The doping leads to distortion in catalyst structure which may result into enhanced catalytic activity. Increased surface area and small diameter as compared to bacterial cells, are linked to higher catalytic activity since more reaction sites are made available. This finding is consistent with the antibacterial activity of Au nanoparticle reported by Wahab et al., [45]. The Au nanoparticle with a small diameter of  $\sim 10 \text{ nm}$  -  $15 \text{ nm}$  exhibited a strong tendency to enter into cells very easily.

Table 1 BET Surface area of LKFO perovskite nanoparticles

Sr.No	Catalyst Composition	Surface Area - $\text{m}^2/\text{g}$
1	$\text{LaFeO}_3$	8.82
2	$\text{La}_{0.9}\text{K}_{0.1}\text{FeO}_3$	11.46
3	$\text{La}_{0.8}\text{K}_{0.2}\text{FeO}_3$	12.62
4	$\text{La}_{0.7}\text{K}_{0.3}\text{FeO}_3$	14.96

## Antibacterial Investigation

### Evaluation of antibacterial activity by well-diffusion method

As described previously, bacteria were cultured and the perovskite nanoparticles with varied concentrations (50, 100, 200, 400 ppm) were poured into the punched wells. After 24 h incubation at 37 °C, the zone of clearance around the wells, indicating inhibition was observed in Figure 3 and Figure 4.

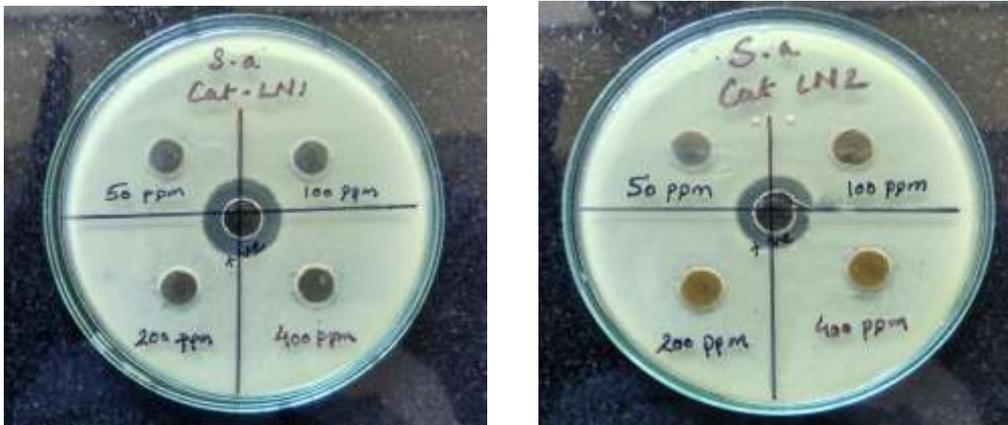


Figure 3. Zone of Inhibition shown by *S. aureus* MTCC 96 against (a)  $\text{La}_{0.9}\text{K}_{0.1}\text{FeO}_3$  and (b)  $\text{La}_{0.8}\text{K}_{0.2}\text{FeO}_3$  perovskite nanoparticles using well diffusion method

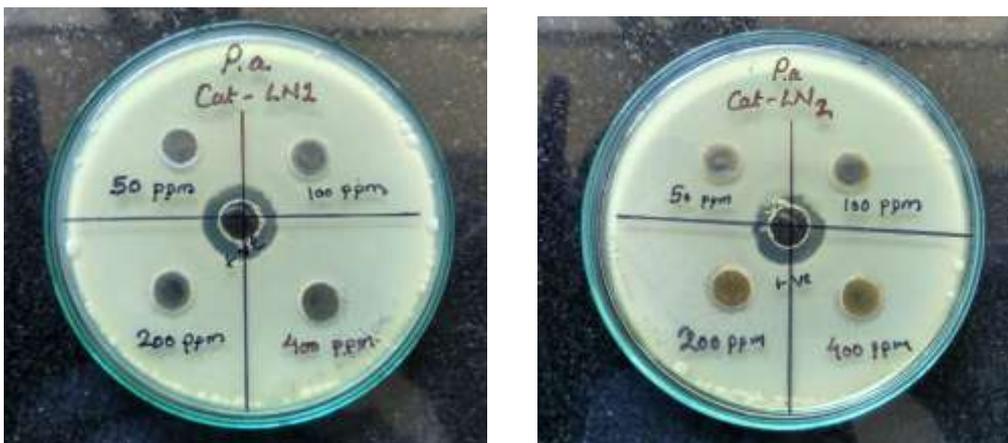
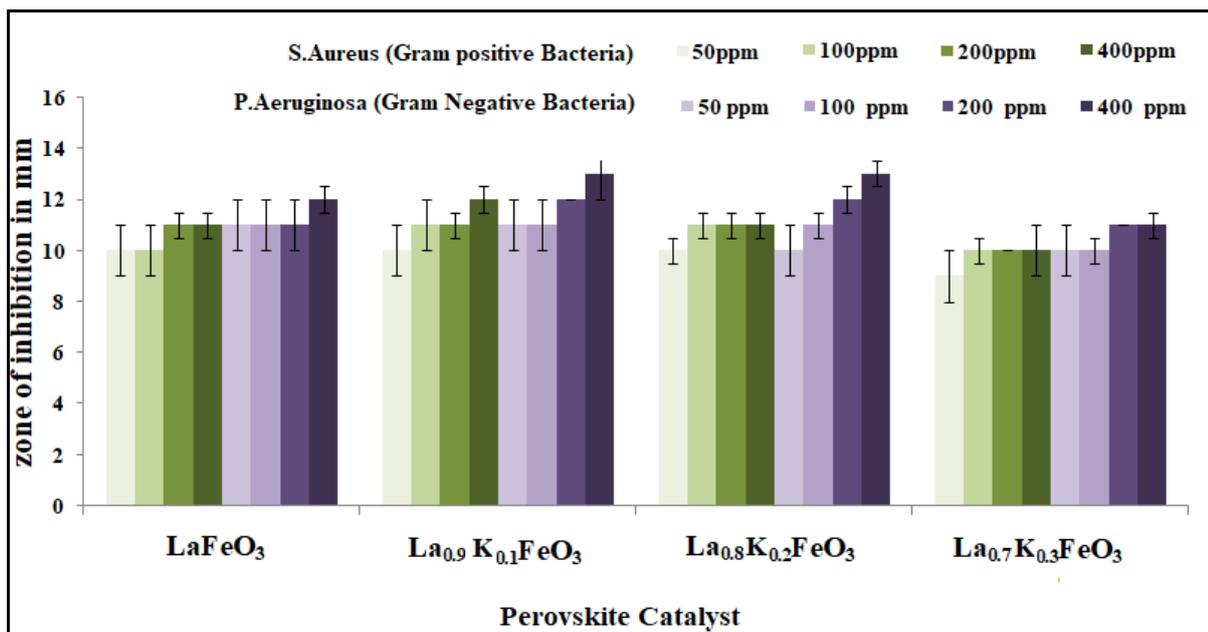


Figure 4. Zone of Inhibition shown by *P.aeruginosa* MTCC 1688 against (a)  $\text{La}_{0.9}\text{K}_{0.1}\text{FeO}_3$  and (b)  $\text{La}_{0.8}\text{K}_{0.2}\text{FeO}_3$  perovskite nanoparticles using well diffusion method.

Both the selected pathogenic cultures were found to be sensitive towards the nanoparticles for the concentrations used as shown in Figure 5. The results obtained in this study are in agreement with Mohseni et al., and Azam et al., [13, 36]. The inhibitory zone around the well periphery shows effective reduction of bacteria concentration by  $\text{La}_x\text{K}_{1-x}\text{FeO}_3$  perovskite materials. The antibacterial activity was maximum at  $x=0.9$  i.e  $\text{La}_x\text{K}_{1-x}\text{FeO}_3$  stoichiometry composition.



**Figure 5. Evaluation of zone of inhibition for the perovskite nanoparticles against the S.aureus and P. Aeruginosa**

**Note :** Ciprofloxacin (10 ppm) is used for positive control gives zone of inhibition (mm)  $17 \pm 1.0$  and  $18 \pm 1.0$  against S.Aureus (Gram Positive Bacteria) and P.Aeruginosa (Gram Negative Bacteria) respectively.

#### MIC and MBC determination

The MIC values for the LaFeO<sub>3</sub> nanoparticles were 200 mg/ml for S.aureus MTCC 96 and 100 mg/mL for P.aeruginosa MTCC 1688. Varying the stoichiometric ratio of potassium led to a change in the MIC value obtained, with least MIC observed for La<sub>0.9</sub>K<sub>0.2</sub>FeO<sub>3</sub> followed by La<sub>0.8</sub>K<sub>0.3</sub>FeO<sub>3</sub> < La<sub>0.7</sub>K<sub>0.3</sub>FeO<sub>3</sub> perovskite nanoparticle (Table 2). The MIC values for La<sub>0.9</sub>K<sub>0.2</sub>FeO<sub>3</sub> was 50 and 100 mg/mL against S.aureus MTCC 96 and P.aeruginosa MTCC 1688 respectively, which are in reasonable agreement with Mohseni et al., [36]. However, the MIC values reported by Ruparelia et al. [14], for Ag and Cu nanoparticles against S.aureus is 120 mg/ml and 140 mg/ml, respectively, which was found to be more than observed value of our present research work. Overall lesser MIC values were obtained for the catalysts against Gram negative P.aeruginosa MTCC 1688 than against Gram positive S.aureus MTCC 96. The mechanism of the bactericidal effect of La<sub>x</sub>K<sub>1-x</sub>FeO<sub>3</sub> perovskite nanoparticles against bacteria is not very clear. The probable mechanism could be because of better adsorption of the nanoparticles owing to the cationic charge on them. This results in enhanced interaction with Gram negative cell surface due to the presence of high lipopolysaccharide with negatively charged carboxylic group and less thickness of peptidoglycan layer, as opposed to Gram positive organism which have thicker layer of peptidoglycan [17, 18]. Minimum bactericidal concentration of the La<sub>0.9</sub>K<sub>0.2</sub>FeO<sub>3</sub> perovskite nanoparticles for S.aureus MTCC 96 and P.aeruginosa MTCC 1688 was 100 mg/mL, making it the potential antibacterial agent (Table 2).

**Table 2 Minimum Inhibitory (MIC) and Bactericidal (MBC) Concentration of perovskite Nanoparticles**

Sr.No	Catalyst Composition	Bacteria	MIC (ug/ml)	MBC (ug/ml)
1	LaFeO <sub>3</sub>	<i>S. aureus</i> MTCC 96	200	400
		<i>P.aeruginosa</i> MTCC 1688	100	200
2	La <sub>0.9</sub> K <sub>0.1</sub> FeO <sub>3</sub>	<i>S. aureus</i> MTCC 96	50	100
		<i>P.aeruginosa</i> MTCC 1688	100	100
3	La <sub>0.8</sub> K <sub>0.2</sub> FeO <sub>3</sub>	<i>S. aureus</i> MTCC 96	100	100
		<i>P.aeruginosa</i> MTCC 1688	100	100
4	La <sub>0.7</sub> K <sub>0.3</sub> FeO <sub>3</sub>	<i>S. aureus</i> MTCC 96	200	400
		<i>P.aeruginosa</i> MTCC 1688	200	400

## Conclusion

The results showed that MIC values for *S.aureus* and *P.aeruginosa* were in the range of 50 – 200 µg/mL and the least MIC value was obtained for La<sub>0.9</sub>K<sub>0.2</sub>FeO<sub>3</sub> perovskite nanoparticles. Minimum bactericidal concentration (MBC) was also evaluated and showed that the growth of *S.aureus* and *P.aeruginosa* was above 100 µg/mL perovskite nanoparticle concentration. Thus on the basis of well diffusion assay and 96 well technique for assessing antimicrobial activity, La<sub>x</sub>K<sub>1-x</sub>FeO<sub>3</sub> (x= 0.1, 0.2, 0.3) Perovskite nanoparticles showed good bactericidal potential. Present results indicate that La<sub>x</sub>K<sub>1-x</sub>FeO<sub>3</sub> (x= 0.1, 0.2, 0.3) perovskite nanoparticles were effective against both Gram-positive and Gram-negative bacterial strains with superior antibacterial effects on gram negative bacteria. Thus, high antibacterial activity of LKFO reflects the use of an inexpensive nanomaterial which could be a potential antibacterial agent in the waste-water treatment. The scope of application could be further extended to investigate antibacterial activity of various other species of bacteria.

## Acknowledgements

The authors would like to thank Department of Chemistry, G.N.Khalsa College, Mumbai for helpful discussion and providing the necessary lab facility and other support. The Authors are thankful to Icon Analytical Laboratory, Mumbai for SEM facility and Department of Metallurgical Engineering & Material Science (MEMS), IIT- Bombay for XRD facility.

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