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## Antibacterial Activity of Photocatalyst $\text{ZnFe}_2\text{O}_4/\text{TiO}_2$ Composite on *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*)

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**Abstract Background:** Heterogeneous photocatalysis has emerged as an alternative effective technique of bacterial inactivation using uv/visible light irradiation. Present study was designed to determine Antibacterial activities of Neem extract-mediated  $\text{ZnFe}_2\text{O}_4/\text{TiO}_2$  composite against some species of bacteria strains *i.e.* *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) using standard well-diffusion method. **Methodology:** Pure culture of the test bacteria was inoculated on nutrient agar (NA) plates by spread plate technique. One ml of the test solutions containing composite were added with the aid of a new sterile syringe to the wells bored on bacterial lawn to ensure the spread of the inoculums on the entire well and incubated at 37 °C for 24 h. Observations were recorded for presence of zone of inhibition and their size of the zones The  $\text{ZnFe}_2\text{O}_4/\text{TiO}_2$  composite was administered into 6 holes of the well with a varying concentration of  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  using sterile syringe. Likewise, ciprofloxacin (30 mg/ml) were administered into two additional holes serving as controls respectively. *Escherichia coli* (*E. coli*) and *S. aureus* were selected as the target microorganism. **Results:** the results obtain shows that the largest zone of inhibition (31mm) was obtained for *E. coli* and (24mm) for *S. aureus* using  $\text{ZnFe}_2\text{O}_4/\text{TiO}_2$  composite. Therefore it is evident from the result of this study that *E. coli* was more susceptible to the  $\text{ZnFe}_2\text{O}_4/\text{TiO}_2$  compared to *S. aureus*. The effect produced by the standard (ciprofloxacin, 1.5 mg) as control.

**Keywords** Antibacterial activities, zone of inhibition, ciprofloxacin

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### Introduction

Safe drinking water supply is a very important aspect regarding human health, and water disinfection has become an urgent need in present world due the microbes contaminating water. According to World Health Organization (WHO), reported that more than one billion people throughout world do not have access to safe drinking water, and about 3.4 million people, mostly children are dying annually with diseases associated with intake of contaminated water. Pathogens, hazardous chemicals and lack of water sanitation are the major factor of causing severe health related problems in human beings [1]. It has been reported that about 48% acute respiratory infections and diarrheal diseases was found in some south-east Asian countries, and consequently, around 3.07 million deaths are occurring every year. These countries having the highest incidence of the water born diarrhea diseases, causing about 60,000 deaths annually are Bangladesh, India, Indonesia, Myanmar and Nepal [2]. Water withdrawal (%) of total available water indicated a global situation of water (shortage) stress. Some areas of the world would experience water stress as defined by a high percentage of water withdrawal compared to total available water. People in these areas have

lack of access to sufficient and clean water. Water stress levels in 19 countries were severe in 1995. According to estimation, by 2025 more than 32 countries will face severe lack of water, more 3 billion people (around 40% of the global population) will live in water stress areas, most of them will be from China and India. The water withdrawal problem (more than 40%) of world will be increased up to 188% and water withdrawal percentage will also increase from 10-20% to 20-40%. i.e the global water stress situation will become significantly more serious by 2025 than water stress level in 1995. generally, this water stress is greatest in areas where very low precipitation (major deserts) occurs or having large population density (Editors, 2013). According to United Nations Development Programme (UNDP), 11% of the world's population (700 million people) lives under water stress with a per capita water supply below 1,700 m<sup>3</sup>/year [3]. The water crisis may also affect the food production and ability of feeding the next generation. Therefore, presence of microbial pathogens in water must be treated before human consumption. However, some conventional techniques such as chlorination, ozonation, and chloramination were used for many decades and have the disadvantage of discharging some disinfectious by products in treated water, which are reported as potential carcinogens and mutagens. These treatment techniques are not acceptable for the drinking water purification systems, Due to negative health issues of disinfections by products on human [4]. Therefore, there is need to develop promising and viable disinfection processes for providing safe drinking water to the human society.

Heterogeneous photocatalysis has emerged as an alternative effective technique of bacterial inactivation using uv/visible light irradiation [5]. It was firstly reported by Matsunaga [6] Killing of Gram-negative, Gram-positive bacteria, yeast, and green algae using TiO<sub>2</sub>-Pt photocatalyst in presence of UV light. Thereafter, photocatalysts were widely been used in the purification and disinfection of pathogenic microorganism infected water, these microorganisms may be prions, coccidian, bacterial strains, mycobacteria, viruses, fungi, yeast etc. [7]

Photocatalysis became renowned process for the disinfection of water, due to many benefits such as high efficiency to kill the microorganisms without need of any extra chemical oxidant, chemical stability, negligible toxicity, saves energy and cost-effectiveness [4]. Most of the photocatalysts works better in the presence of UV light only, but TiO<sub>2</sub> modification can extend its application to visible light irradiation. Doing of TiO<sub>2</sub> with noble metals, oxides or non-metals are found to increase the photocatalytic activities of titanium dioxide [7]. Due to doping, the bacterial inactivation of photocatalysts are able to work in presence of visible light in the same rate with UV illumination [7], and it appears that the future of photo-disinfection catalyst is bright and can be applied to disinfect the water for drinking purpose.

Therefore, there is need to develop easy use of novel visible light responsive photocatalytic water treatment technology.

### Visible-Light Responsive Photocatalyst

Heterogeneous photocatalysis has been considered a novel and effective technique of water disinfection technology. Upon irradiation with photons of UV light ( $\lambda \leq 385$  nm) on the photocatalyst (e.g. TiO<sub>2</sub>), electron will be promoted from the valance band (VB) to conductive band (CB) and creates an electron-hole pair. These photogenerated electrons and holes react with oxygen molecules with water adsorbed on the TiO<sub>2</sub> surface to produce reactive oxygen species (ROS). ROS can oxidize the organic substances and inactivate the bacteria as well as other pathogenic microorganisms present in water. However, the most of the pure form of photocatalyst can work only with UV light but this limits its practical applications [8]. Hence, visible-light responsive photocatalyst is more attractive and effective for disinfection as compared to UV light driven photocatalyst [9]. By doping non-metals element into the matrix of TiO<sub>2</sub> can expand its application to the spectrum of visible light, because doping reduces the stimulation band gap and increased the efficiency of titanium dioxide [7]. Authors have successfully synthesized a visible light responsive photocatalyst in laboratory conditions via doping of nitrogen in the structures of titanium dioxide and it is designated as nitrogen doped titanium dioxide (N-TiO<sub>2</sub>). This visible light responsive N-TiO<sub>2</sub> photocatalyst has shown the significant potential for the disinfection of water and may be employed at a large scale



of water treatment plants in the future. This advantage of using visible light responsive  $\text{ZnFe}_2\text{O}_4/\text{TiO}_2$  photocatalyst reveals its economic feasibility for heterogeneous photocatalysis.

### Study Area

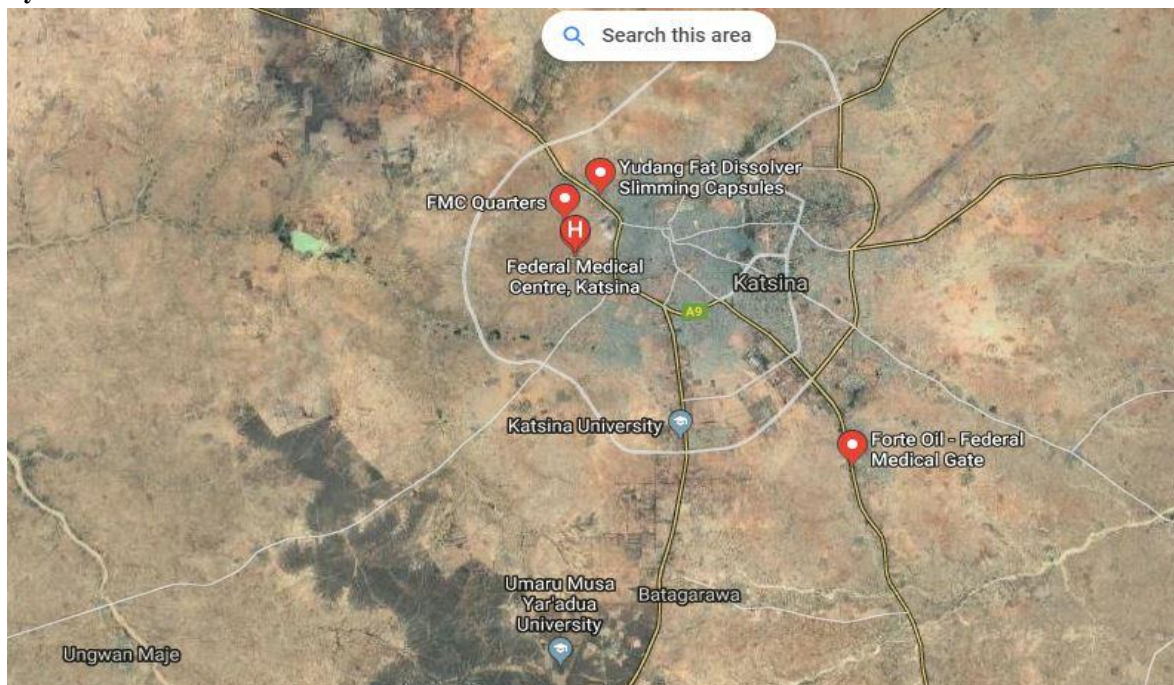


Figure 1: Map showing the study location (source: [www.google.com/maps/](http://www.google.com/maps/))

### Materials and Method

#### Sample collection/ Bacterial isolates

The sample was obtained from federal medical Centre Katsina and transported directly to Umaru Musa Yar'adua University, microbiology laboratory for purity test; the isolates are *Escherischia coli* and *S. aureus*. It was maintained on nutrient agar slants at 4 °C till use.

#### Sample preparation and media serial dilution

Samples were prepared using serial dilution method. 1 g of the  $\text{znFe}_2\text{O}_4/\text{TiO}_2$  sample was dispensed in a test tube containing  $1\text{cm}^3$  of distilled water, as a stock solution, which later transferred into a series of six test tubes containing  $9\text{cm}^3$  of distilled water and the series of concentration were given as  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  ( $\text{gml}^{-1}$ ) [10].

#### Well preparation and inoculation

Reagents used in all tests were of analytical quality. Deionised water was used to prepare all microbiological media. Required amount of Nutrient agar media was dispensed in the conical flask containing distilled water; it was shaken for absolute homogeneity and later autoclave at 121 °C for 15 minutes. The Nutrient agar media was dispensed in petri dishes for solidification [10]

### Results and Discussion

#### Antibacterial activity of $\text{ZnFe}_2\text{O}_4/\text{TiO}_2$ composite

Antibacterial activities of Neem extract-mediated  $\text{ZnFe}_2\text{O}_4/\text{TiO}_2$  composite were assayed using standard well-diffusion method. Pure culture of the test bacteria was inoculated on NA plates by spread plate technique. One ml of the test solutions containing composite were added with the aid of a new sterile syringe to the wells bored on



bacterial lawn to ensure the spread of the inoculums on the entire well and incubated at 37 °C for 24 h. Observations were recorded for presence of zone of inhibition and their size of the zones. The ZnFe<sub>2</sub>O<sub>4</sub>/TiO<sub>2</sub> composite was administered into 6 holes of the well with a varying concentration of 10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup> (gml<sup>-1</sup>) using sterile syringe. Likewise, ciprofloxacin (30 mg/ml) were administered into two additional holes serving as controls respectively.

The efficiency of ZnFe<sub>2</sub>O<sub>4</sub>/TiO<sub>2</sub> composite disinfection of water has been tested, *Escherichia coli* (*E. coli*) and *S. aureus* were selected as the target microorganism, and this bacteria causes diarrhea diseases in humans after taking it infected water.

During the experiment, the effect of ZnFe<sub>2</sub>O<sub>4</sub>/TiO<sub>2</sub> dosages on the photocatalytic inactivation of *E. coli* and *S. aureus* were observed using 1.0 g/L ZnFe<sub>2</sub>O<sub>4</sub>/TiO<sub>2</sub> at six respective initial bacterial concentrations as 10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup> (gml<sup>-1</sup>). All the inhibition zones were shown in Table 1.

### Zone of Inhibition

After 24 hours of incubation. Zone of inhibition were determined which was the area inhibited by the ZnFe<sub>2</sub>O<sub>4</sub>/TiO<sub>2</sub> composite that prevent the bacteria from growing and the clear zones of inhibition (mm) were measured using meter ruler.

**Table 1:** Mean zone of inhibition (mm) of *E. coli* and *S. aureus* at various concentrations

Concentrations (gml <sup>-1</sup> )	Zone of inhibition (mm)	
	<i>E. coli</i>	<i>S. aureus</i>
10 <sup>-1</sup>	31	24
10 <sup>-2</sup>	26	16
10 <sup>-3</sup>	20	15
10 <sup>-4</sup>	12	09
10 <sup>-5</sup>	12	NI
10 <sup>-6</sup>	09	NI
Control	30	22

N.B; the values recorded are duplicate readings, NI means no inhibition

### Conclusion

In summary of present study, an effort has been made to develop a ZnFe<sub>2</sub>O<sub>4</sub>/TiO<sub>2</sub> composite, which is able to work efficiently for antimicrobial activity. ZnFe<sub>2</sub>O<sub>4</sub>/TiO<sub>2</sub> composite was synthesized in laboratory conditions and examined for the inactivation of *E. coli* and *S. aureus* bacteria. Inhibition zones for the inactivation were shown in Table for both *E. coli* and *S. aureus* with 1.0 g/L ZnFe<sub>2</sub>O<sub>4</sub>/TiO<sub>2</sub> dosage.

The prepared composite were screened for antimicrobial activity by well diffusion method. It was observed that the prepared composite has antibacterial potential against two pathogenic bacteria (gram +ve and gram -ve). Inhibition of microbial growth in terms of inhibition zones around the test wells in a bacterial lawn was observed and compared with no zone of inhibition under control conditions. It is observed that the largest zone of inhibition (31 mm) was obtained for *E. coli* and (24 mm) for *S. aureus* using ZnFe<sub>2</sub>O<sub>4</sub>/TiO<sub>2</sub> composite. Therefore it is evident from the result of this study that *E. coli* was more susceptible to the ZnFe<sub>2</sub>O<sub>4</sub>/TiO<sub>2</sub> compared to *S. aureus*. The effect produced by the standard (ciprofloxacin, 1.5 mg) as control.

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