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Zinc Oxide Supported onto Biogenic Silica from *Gigantochloa atroviolacea* Leaves: Preparation, Characterization and Antibacterial Activity

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Bamboo rod is a well known raw material utilized to make houses, papers, handicrafts, chopsticks and medicines. On the other hand, the utilzation of leaves, which are often considered as garbages, receive less attentions. Even though, bamboo leaves are good sources of SiO_2 or silica. This work reports the preparation of zinc oxide (ZnO) supported onto biogenic silica (SiO_2) from leaves of *Gigantochloa atroviolacea*, characterization and the antibacterial activities of ZnO@SiO_2 against *Staphylococcus epidermidis*. The ZnO was supported onto biogenic SiO_2 by using impregnation method of SiO_2 in zinc salt [$Zn(NO_3)_2{\cdot}6H_2O$] solution. A combination XRD and SEM-EDX techniques were used to confirm the formation of ZnO on ZnO@SiO_2. Investigations indicate that the as prepared ZnO@SiO_2 revealed significant antibacterial activity against *Staphylococcus epidermidis* bacterial strain.

Keywords: ZnO@SiO_2, Biogenic silica, *Gigantochloa atroviolacea*, *Staphylococcus epidermidis*.

INTRODUCTION

Microorganisms may give beneficial or harmful effects on the environment regarding to the human being. It is necessary to control microorganisms harmful effects by inhibiting their growth. Antimicrobial agents are chemical compounds having potential to inactivate or inhibit the growth of microorganism [1]. The antimicrobial agents have vast applications in various fields including food preservation and packaging, medicine, water disinfection, textile fabrics and hospital implants [2,3].

Zinc oxide is one of attractive metal oxides and has been used as an active constituent in ointment, creams and lotions for skin treatments owing to its antibacterial properties [4-6]. Zinc oxide is also widely used to treat dermatitis, itching due to eczema, diaper rash and acne [7]. It is used in products such as baby powder and barrier creams to treat diaper rashes, calamine cream, antidandruff shampoos and antiseptic ointments [8,9].

Zinc oxide nanoparticles indicate more antimicrobial activities than large particles, since the high surface-to-volume ratio of nanoparticles allows for better interaction with bacteria [10,11]. Zinc oxide nanoparticles have been shown to have a wide range of antibacterial activities including major food-borne pathogens like *Escherichia coli*, *Salmonella*, *Listeria monocytogenes* and *Staphylococcus aureus*. The *Staphylococcus aureus* family contributes to 65-90 % of all *Staphylococci* recovered from human aerobic flora. It has developed resistance to many common antibiotics such as methicillin, novobiocin, clindamycin and benzyl penicillin [12].

Currently there is a little information available on their antibacterial effect against species of *Staphylococcus epidermidis* [13-15]. *Staphylococcus epidermidis* is a Gram-positive and a true opportunistic pathogen. It is one of the leading pathogens of nosocomial infections. Those most susceptible to infection are intravenous drug users, newborns, elderly and those using catheters or other artificial appliances [16].

Zinc is an essential element for microorganisms and higher organisms because it involved in many vital cellular reactions at its low endogenous concentrations [17-19]. Zinc concentration is regulated under physiological conditions by several transporters, so that Zn^{2+} ions are essentially non-toxic to higher organisms [20,21]. Homeostasis regulates zinc uptake by cells, but it does not control zinc adsorption to cell membranes however, increase in Zn^{2+} concentrations above optimal levels perturbs Zn^{2+} homeostasis and allows entry of Zn^{2+} inside cells,

so that zinc starts being cytotoxic to prokaryotes above a concentration of 10^{-4} M. Therefore, Zn^{2+} displays an antimicrobial activity and could act as either antibacterial or antifungal agent [22,23].

In dermatological products, zinc ions are interesting biocides and/or antimicrobial preservatives provided that high enough concentrations of Zn^{2+} are generated. The previously mentioned zinc salts can be simply dissolved in the aqueous medium. An alternative is solid powder such as ZnO particles that release Zn^{2+} in the aqueous medium. It is indeed recognized that part of the antimicrobial activity of ZnO particles originates from their ability to partially dissolve in aqueous media. Release of Zn^{2+} would contribute to the global antimicrobial properties of this inorganic powder [24,25].

However, zinc oxide nanoparticles are much more effective in suppression of microorganism growth. Furthermore, at nanoscale the properties of ZnO particles are widely changed, enhancing their efficacy in inhibiting the growth of bacteria [5,26,27]. The antimicrobial potential of metal oxide nanoparticles has been recognized to their smaller size and higher surface to volume ratio, which enables them to closely bind with microbial strain but it does not due to the discharge of metal ions in solution [5,28].

Nanotechnology is getting advancement particularly the potential to prepare metal oxide nanomaterials of specific shape and size which leads it in the development of new antibacterial agents [29,30]. Different methods have been used to synthesize nanoparticles like sol-gel method [31], surfactant mediated method [32], deposition-precipitation method [33], anodization method [34], wet-oxidation method [35], microwave-assisted combustion [36], electrodeposition and sonication [37]. Zinc oxide nanoparticles synthesized by any of these methods are used against bacterial strains by adopting various protocols. The catalytic activity and antimicrobial properties of nanoparticles can be enhanced by doping the nanoparticles with various methods. In this study, we have designed method to prepare zinc oxide nanoparticles supported onto a stable-wide surface biogenic SiO_2 extracted from the leaves of *Gigantochloa atroviolacea*. The evaluation of antibacterial effects of ZnO against *Staphylococcus epidermidis* is reported.

EXPERIMENTAL

Biogenic silica was isolated by calcining 100 g of dry *Gigantochloa atroviolacea* leaves at 650 ºC for 4 h, followed by air cooling and washing with 0.1 M HCl solution. The ZnO@SiO_2 was prepared by mixing 1.9848 g of biogenic silica and 0.0152 g of zinc nitrate [$Zn(NO_3)_2{\cdot}6H_2O$ (Merck)] (0.25 mol % zinc to silica) in 5 mL of distilled water. The mixture was then stirred for 2 h at which time 5 mL absolute ethanol was added and the solid was isolated by filtration and air dried. The dry sample was then calcined at 800 ºC for 4 h, resulting in biogenic silica supported zinc oxide (ZnO@SiO_2). The catalysts with 0.5 mol % of zinc were prepared by the same procedure except using 0.0304 g of zinc nitrate.

The ZnO@SiO_2 photocatalysts were characterized by X-ray diffraction (XRD Lab-X Type 6000 Shimadzu, Japan) using Cu Kα radiation (λ = 1.5418 Å) over the angular range $10^\circ \le 2\theta \le 90^\circ$ at a scan speed of 0.02 deg s^{-1}. Scanning electron micrographs were measured using a Jeol JED-2300 instrument.

Antibacterial activity of ZnO@SiO_2 was studied against a bacterial strain *Staphylococcus epidermidis*. The bacterial culture, nutrient broth (Oxoid, UK) 100 mL was prepared in Erlenmeyer flask contained glass beads. The pH of broth was adjusted to 7.4 by addition of buffer solution, and autoclaved at 121 ºC for 15 min. The mixture was allowed to cool in open air. The broth was inoculated with 100 µL *Staphylococcus epidermidis* form stored bacterial culture. Growth medium was incubated in a shaker at 37 ºC for 24 h to get 5×10^9 cells per mL.

Nutrient was prepared by mixing agar in distilled water. The nutrient and 10 mm discs of wicks paper were sterilized in autoclave and allowed to cool in air. The nutrient was inoculated with 50 µL fresh *Staphylococcus epidermidis* culture and sterilized discs were poured with synthesized ZnO@SiO_2 and spread in petri dishes with positive control in the other side. The petri-dishes incubated at 37 ºC for 24 h. Zones were measured with zone reader. The different levels of zone of inhibition were measured every 3 h for 24 h for determining the antimicrobial activity of synthesized ZnO@SiO_2.

RESULTS AND DISCUSSION

The study of ZnO@SiO_2 prepared using biogenic silica from *Gigantochloa atroviolacea* was reported in this work. The ZnO@SiO_2 was also tested for antimicrobial activity against Staphylococcus epidermidis. The ZnO@SiO_2 was investigated by X-ray diffraction. Fig. 1 shows the XRD pattern for ZnO@SiO_2. The difractograms indicate the presence of SiO_2 (Fig. 1a-b). The weak-broad XRD line of (002) (Fig. 1b) can be considered as small-amorf satelites of ZnO (JCPDS No. 00-036-1451). The crystal nature of ZnO is wurtzite hexagonal which is similar as reported by Darroudi *et al.* [38] and Zak *et al.* [39].

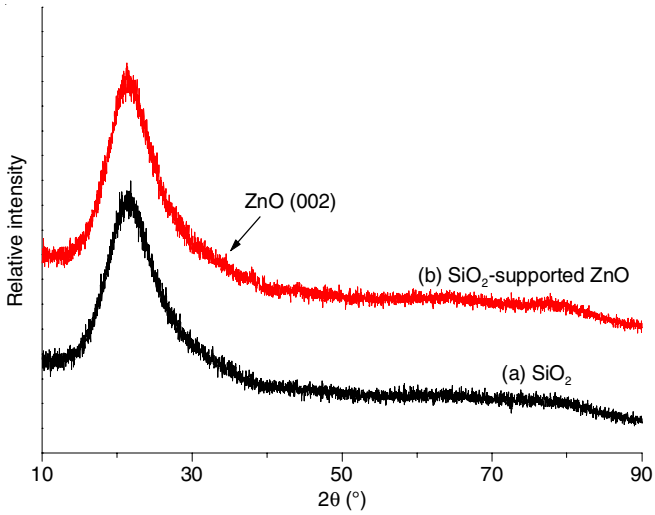

Fig. 1. X-ray diffraction pattern of (a) SiO_2 and (b) ZnO@SiO_2

In order to verify the results of X-ray diffraction analysis, ZnO on the surface of SiO_2 were examined by TEM analysis. From TEM analysis (Fig. 2), ZnO is observed as rounded in shape and is observed having diameter about 200 nm, which can be catagorized as nanoparticles. However, Santhaveesuk *et al.* [40] successfully prepared smaller ZnO nanoparticles, that are 47 nm in size, *via* the coprecipitation method using zinc nitrate and sodium hydroxide as raw materials.

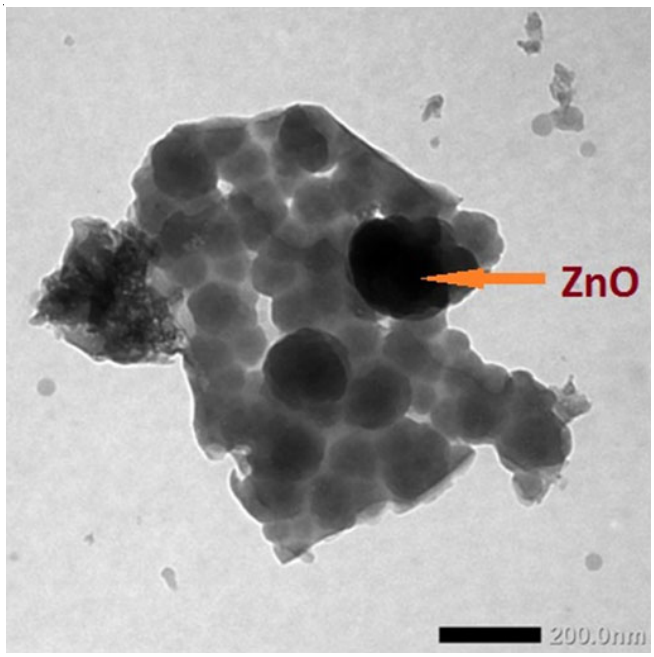

Fig. 2. TEM micrograph of ZnO@SiO_2. The arrow indicates ZnO crystallites

The SEM micrograph represents the morphology of ZnO@SiO_2 (Fig. 3). The SiO_2 shows irregular form and surface and has a size of between 4 to 30 µm. Holes with the diameter of around 2 µm are observed over the surfaces. The presence of ZnO is difficult to be located, but it is believed that the iregular in shape of ZnO is placed on the surface of SiO_2 and this is supported by the EDX analysis (Fig. 4). Fig. 4 confirms the presence of ZnO on the sample with sharp signals of Zn (ZnLα_1, ZnKα_1, ZnKβ_1), along with O and Si lines. An EDX analysis indicates that the proportion of Zn to Si is expected.

The antibacterial effect of prepared ZnO@SiO_2 is studied on *Staphylococcus epidermidis*. The ZnO@SiO_2 inhibition zone value and the control antibiotic chlorofenicol are given in Table-1.

The zone of inhibition of ZnO@SiO_2 is about 50 % compared to the control antibiotic chlorophenicol. This indicates that ZnO@SiO_2 is quite powerful to inhibit the development of *Staphylococcus epidermidis*. The inhibition is maximum

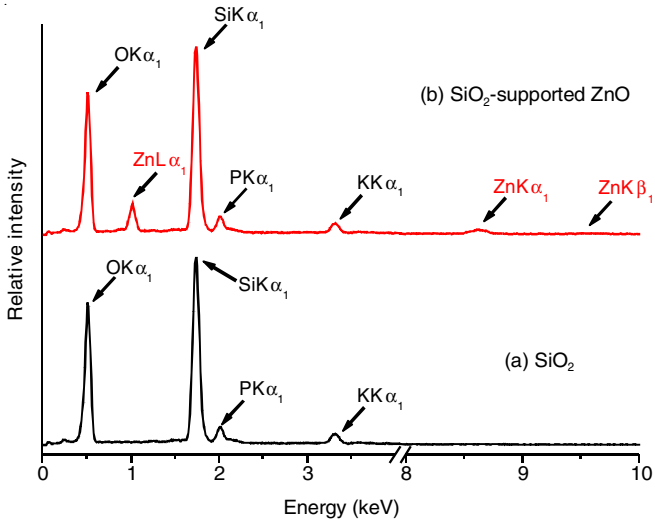

Fig. 4. EDX pattern of (a) SiO_2 support and (b) ZnO@SiO_2

TABLE-1
ZnO@SiO_2 INHIBITION ZONE AND THE CONTROL ANTIBIOTIC CHLOROFENICOL

Observation time (h)	Zone of inhibition (mm)		
	Chlorofenicol (control)	SiO_2	ZnO@SiO_2
3	18.7	0	9.3
6	18.0	0	9.1
9	19.7	0	8.2
12	18.6	0	8.0
15	19.4	0	8.5
18	21.2	0	8.0
21	20.4	0	7.9
24	19.9	0	7.8

after 3 h of the introduction of ZnO@SiO_2. The SiO_2 is absent in bacteria inhibition, and this indicates that bacteria inhibitions is occured as result of ZnO supported on the surface of SiO_2. Hence, green synthesis of SiO_2 can also be used as good support for ZnO antibacterial agent.

Conclusion

The ZnO@SiO_2 nanoparticles have been synthesized and characterized. The characterization by using XRD, SEM, EDX

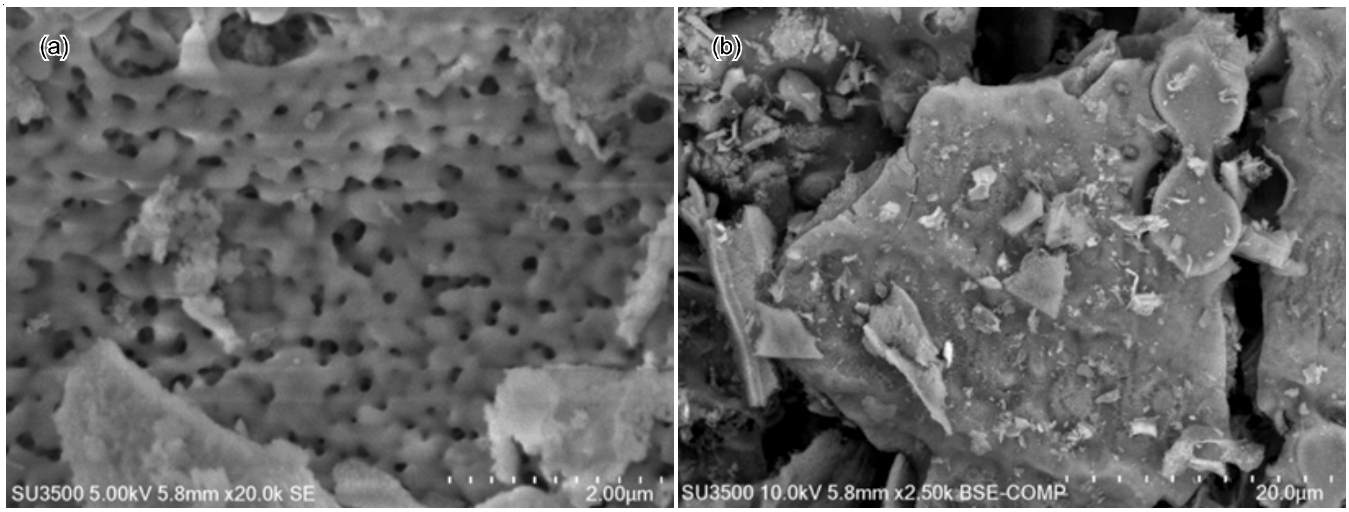

Fig. 3. SEM micrograph of synthesized ZnO@SiO_2, (a) 20.000 X, and (b) 2.500 X magnifications

and TEM methods confirmed the formation of ZnO@SiO_2. The zone of inhibition in the antimicrobial screening indicated that synthesized ZnO@SiO_2 has efficient antimicrobial activity against *Staphylococcus epidermidis*.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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