In situ Formation of Zinc Oxide on Bamboo Bleached Pulp in Preparation of Antibacterial Paper: Effect of Precursors Addition

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An approach of green *in situ* synthesis single-step method was applied to produce antibacterial paper. The objective was to investigate the effect of precursor addition on the formation of zinc oxide particles using an *in situ* single-step method. Zinc chloride concentrations of 0.1, 0.3, 0.5, and 0.7 M were prepared and added into a solution of algae extract and bamboo pulp. The prepared pulps were tested and made into handsheets using a papermaking machine based on TAPPI T205 (2006). Morphological observation of treated papers was conducted using a field emission scanning electron microscope (FESEM). An average of 400 to 570 nm zinc oxide spherical-shaped particle was observed on the fibers of paper. The percentage of element composition of the treated paper were 15.08% to 34.08% of zinc and 17.45% to 32.59% of oxygen captured *via* scanning electron microscopy with energy dispersive X-ray (SEM-EDX) analysis. The crystallinity test was performed using X-ray dispersion (XRD). A higher percentage of precursors exhibited a more amorphous structure. A measurement of more than 30% increment of inhibition zone was obtained from 10.00 to 25.00 mm against *S. aureus*, *S. choleraesuis*, and *E. coli.* Precursors addition of more than 0.3 M would have the most potential to enhance the growth of zinc oxide *via in situ* preparation, hence providing better antibacterial properties of the prepared papers.

Keywords: Algae; Zinc oxide; Bamboo; Bleached pulp; In situ preparation; Papermaking

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INTRODUCTION

Zinc oxide is a multifunctional mineral due to its physical and chemical properties. It exists as a mineral named zincite and has been commercially synthesized for commercial purpose (Mirzaei and Darroudi 2017). Its non-toxicity and compatibility to human skin has placed zinc oxide in a wide range of applications such as in pharmaceuticals (Pronin *et al.* 2014), paints (Osmond 2019), textiles (Rajendra *et al.* 2010; Belay *et al.* 2020), and the electronic industries (Balogun *et al.* 2020). Among all material particles, zinc oxide is well known for having the most varied particle structures (Kołodziejczak-Radzimska and Jesionowski 2014), especially one-dimensional structures such as needles, ribbons, tubes, wires (Nikoobakht *et al*. 2013), rods (Jaisai *et al.* 2012), prisms (Wirunmongkol *et al*. 2013), sheets (Lu *et al*. 2017), triangles (Nagarajan and Kuppusamy 2013), squares (Mishra and Sharma 2015), and combs (Xu *et al*. 2012). Besides that, zinc oxide appears in two-dimensional and three-dimensional structures. The two-dimensional structures including nanoplate and nanopellets while flower, dandelion, snowflakes, coniferous urchin-like and

polygonal (Zhou *et al*. 2013; Namvar and Rahman 2013) are the zinc oxide shapes that are grouped in three-dimensional structures. The shape, size, and spatial structure can be tailored by adjusting methods during the preparation of zinc oxide. In general, three methods are applied to prepare zinc oxide: (1) chemical, (2) physical, and (3) biological. Controlled precipitation, the sol-gel method, solvothermal and hydrothermal methods, and the method using emulsion and microemulsion environments are categorized as chemical methods (Kolodziejczak-Radzimska and Jesionowski 2014). The biological method, which is regarded as a green method, uses substrates including plant extracts, microorganisms, enzymes, and bacteria.

The biological or green method in processing zinc oxide has attracted many researchers in biosensors, pharmaceuticals, cancer treatment, and magnetic resonance imaging (MRI) areas (Kulkarni and Muddapur 2014). This is due to its eco-friendly, non-toxic, and safe reagent that is preferred in the biologically mediated preparatory method compared to the chemical and physical methods (Bhuyan *et al*. 2015). In addition, efficiency in cost and synthesizing particles without pressure has led to truly green chemistry (Nagarajan and Kuppusamy 2013; Sutradhar and Saha 2015). Equation 1 shows the reaction in zinc oxide formation (Sutradhar and Saha 2015). A study conducted by Jamdagni *et al*. (2016) succeeded in producing 12 to 32 nm of zinc oxide particles in aggregates from *Nyctanthes arbortristis* (flower) extract. This occurred when a bioactive compound contained in biomaterial reduced the metal element to form metal oxide. In examples, this can successfully be achieved by gentle warming and incubation that could activate quinones in *Cyprus sp.* (mesophytes) to reduce its zinc oxide particle size (Kirthi *et al.* 2013). Algae, also known as bionanofactories, can be synthesized nanoparticles with high stability and important sources in producing metallic-nanoparticles (Ramaswamy *et al.* 2016). Macroalgae such as seaweeds that have become part of habitual diet in Asian countries can be classified into three subcategories: *Phaeophyta* (brown seaweed), *Rhodophyta* (red seaweed), and *Chlorophyta* (green seaweed) (Brownlee *et al*. 2012).

$$Zn^{2+} + 2e \rightarrow Zn^{0} + \text{Air } (O_2) \rightarrow ZnO \quad (1)$$

In manufacturing antibacterial paper, a coating technique is usually selected by incorporating antibacterial agent in coating film that is later coated on the paper surface. Another coating technique was applied by Akrami *et al*. (2015). The essential oils from cumin and a thyme-like plant that have antibacterial properties were extracted and applied on paper surfaces and presented good antibacterial activity against food-borne bacteria (Akrami *et al*. 2015). Prasad *et al*. (2010) also applied a coating technique and observed that no bacterial growth was observed for zinc oxide-coated paper. Other than the coating technique, an *in situ* bio-mediated precipitation method was also practiced. A study conducted by El-Samahy *et al*. (2017) produced antibacterial paper by incorporating chitosan and nanocellulose via in situ approaches that resulted in strong antibacterial activity of paper. In terms of applying inorganic material as antibacterial agent, Maślana *et al.* (2021) boosted antibacterial performance of their cellulose-based paper sheet by using titanium dioxide particles. In addition, Li *et al.* (2021) also focused on *in situ* growth of nano-ZnO/GQDs on their cellulose paper to repel against water and bacteria.

Bamboo is a member of a large woody grass family and is a fast-growing plant that can be found naturally in subtropics and temperate zones such as Asia. It is reported that bamboo can grow three times faster than eucalyptus with 1,300 to 1,400 species around the world (Liese and Kohl 2015; Yeasmin *et al.* 2015; Tripathi *et al.* 2018) with 120 species in Asia that include 59 species in Malaysia. The most common bamboo species such as

Semantan (*Gigantochloa scortechicinii*) and Gading (*Bambusa vulgaris*) exhibit excellent strength capacity for building material compared to wood and concrete except concrete has better stiffness (Janssen 1981; van der Lugt *et al.* 2006; Dhanush *et al.* 2021). Other than being a good building material, a strong utensil, and current hygiene instrument as a toothbrush, bamboo is also an ideal material in papermaking (Chen *et al.* 2019; Hammett *et al*. 2001). Bamboo can be applied in producing advanced material such as antibacterial fabric. Zhang *et al*. (2013) improved antibacterial activity of bamboo fabric at 99% performance degree against *S. aureus* and *E. coli* by synthesizing zinc oxide. Another study conducted by Yin *et al*. (2017) improved the porosity properties of bamboo tissue paper's (napkins) surface by incorporating chitosan. In addition, Zhang *et al*. (2017) improvised bamboo antibacterial performance using zinc oxide and graphene oxide. The antibacterial bamboo is also expected to be a potential material in the textile industry.

Looking at the small number of studies that concentrated on the *in situ* bio-mediated preparation of zinc oxide, a study was conducted to be simpler in a single step and easier than the usual method. This study focused on the effect of precursor concentration, which is believed to be a great influencer to zinc oxide *in situ* performance.

EXPERIMENTAL

Raw Materials and Chemicals

The 2 m of Semantan bamboo culms (*Gigantochloa scortechicinii*) were purchased from Sungai Siput, Perak, Malaysia and ground into chips until 2 to 3 cm width and length. Air-dried algae of *Rhodophyta* were acquired from Semporna, Sabah, Malaysia. The chemicals involved in the study were zinc chloride ($ZnCl_2$), sodium hydroxide (NaOH), sodium chlorite ($NaClO_2$), acetic acid (CH_3COOH), and hydrogen peroxide (H_2O_2). All chemicals were purchased from RandM Chemicals (Selangor, Malaysia).

Preparation of Bleached Pulps of Bamboo

These bamboo chips were cooked using a soda pulping technique with 17% sodium hydroxide in a twin digester (GIST, Daejeon, South Korea) at 170 °C for 90 min. After completing the pulping process, the pulp was washed and screened using a Somerville Shive Content Analyser (Frank-PTI, Birkenau, Germany). The next step was the bleaching process, in which delignification (D) and extraction (E) processes as $D_1E_PD_2$ sequences were applied in a 70 °C water bath. The consistency of unbleached pulp was 10% which was placed in a transparent plastic bag containing 2.00% of $NaClO_2$ and 3.00% of CH_3COOH throughout the D_1 stage for 180 min. The bleaching process continued with the extraction step, in which a mixture of NaOH at 1.50% and H_2O_2 at 1.00% was applied to the pulps for 90 min. Finally, the D_2 stage involved the immersion of pulps for another 90 min by adding $NaClO_2$ and CH_3COOH at 1.25% and 3.00% concentrations accordingly. The pulps were washed between bleaching sequences. Prior to further experiment, the bleached pulp was beaten by using a PFI-mill Beater according to the TAPPI T248 (2000) standard method reaching 350 to 400 mL.

Pulp and Paper Characterization

Approximately 500 g of dried algae was washed and oven-dried at 60 °C overnight. The dried alga was ground using a blender to obtain algae powder, which was then sieved with 40-mesh sifter to collect a uniform particle size. Later, 2 g of dried seaweed was heated with 200 mL of distilled water until it boiled before filtering it, of which the effluent was

taken into the next experiment. The preparation of green zinc oxide nanoparticles *via in situ* approaches was conducted by incorporating 2.4 g of bleached pulps in the 100 mL of extract solution. Prior to that, the extract solution was prepared by adding different concentrations of pre-cursor, namely zinc chloride, and heated for 4 h at 50 °C in a water bath. Finally, the samples were rinsed with distilled water and proceeded to the papermaking by using handsheet machine. The papermaking process was referred to the TAPPI T205 (2006), producing a 60 g/m^2 piece of paper. The list of samples prepared in this study is shown as in Table 1.

Table 1. Samples Prepared for the Study

Samples	B1	B3	B5	B7
Concentration of Zinc Chloride	0.1 M	0.3 M	0.5 M	0.7 M

Characterization of Zinc Oxide Nanoparticles and Zinc Oxide Paper

The formation and distribution of zinc oxide on papers was observed using field emission scanning electron microscope (FESEM) (Model: JSM 7600F, JEOL, Tokyo, Japan). The energy dispersive X-ray (EDX) was applied to confirm the elements that attached on the fibers. The properties of zinc oxide-paper were characterized *via* X-ray dispersion (XRD) (PW 3040/60 MPD X'pert High Pro Panalytical, Phillips, Omaha, NE, USA) while antibacterial testing was conducted against three types of bacteria explicitly as *Staphylococcus aureus* (positive strain bacteria), *Salmonella choleraesuis*, and *Escherichia coli* (negative strain bacteria) using the agar well diffusion method (Balouiri *et al*. 2016).

RESULTS AND DISCUSSION

Morphological Observation of Zinc Oxide Paper

Morphological observation of zinc oxide paper is exhibited as micrographs shown in Fig. 1 *via* 500×, 1,500×, and 10,000× magnifications. Flocs of elements were observed on the fiber surfaces of the papers. This was confirmed after spotting the elements using FESEM-EDX, which represented the elements of zinc and oxygen as shown in Fig. 2 and Table 2.

Table 2 exhibits scanning electron microscopy with energy dispersive X-ray (SEM-EDX) analysis of the spotted area for two elements, namely Zn and O, that appeared on paper surfaces. Figure 2 shows the percentage weight of zinc and oxygen for B1, B3, B5, and B7, which ranged from 15.08% to 34.08%, and 17.45% to 32.59%, respectively. Different weight, size, and shape of zinc oxide particles are the outcomes of interactions between bioactive compounds in algae like phenols, and metal atoms from precursor were added into the systems (Shao *et al.* 2004).

The zinc oxide particles were measured with a range of 400 nm to 570 nm. They appeared in spherical form (Fig. 3), as achieved by Vinay and Chandrasekhar (2021), Vijayakumar *et al.* (2018) and Yedurkar *et al.* (2016). Chronologically, zinc oxide particles were synthesized by reducing Zn^{2+} from zinc chloride with the bioactive compounds present in algae extract (Sadatzadeh *et al*. 2018). After *in situ* preparation stage, the pulps were poured into the handsheet machine tank before filtering the pulp slurry to leave a sheet of paper on the wire mesh. Several parts of the elements of zinc oxide were believed to be flushed away, which researchers continue to find ways to protect as much zinc oxide

from heavy dislodgements. As the findings of these preliminary experiments appear very beneficial, the retention of additives will be studied and applied in future experiments.

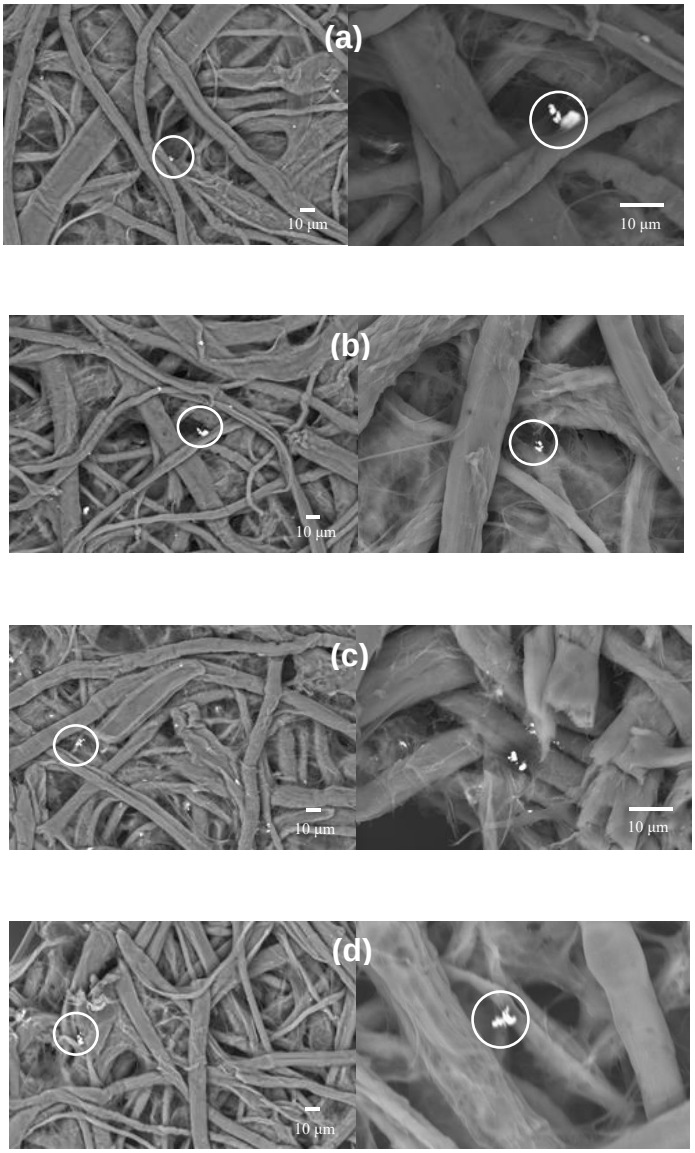
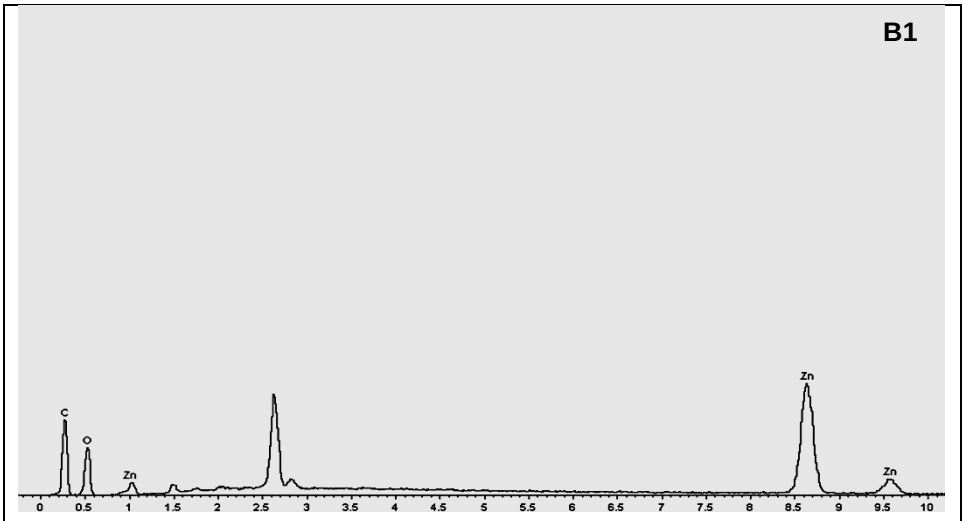


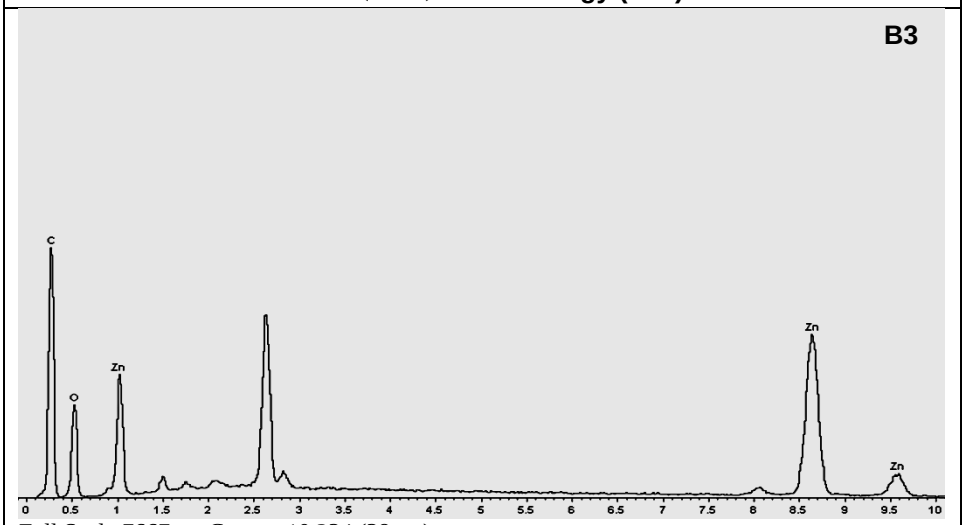
Fig. 1. FESEM micrographs of (a) B1, (b) B3, (c) B5, and (d) B7 with magnification of 500X (left column) and 1,500X (right column); the presence of zinc oxide is shown in the circles.

Table 2. SEM-EDX Analysis of Flocs in Terms of Two Elements that Appeared on Paper Surfaces

Sample	Percentage (%)	
	Zinc	Oxygen
B1	34.08	17.45
B3	23.75	17.72
B5	16.36	28.57
B7	15.08	32.59



Full Scale 7667 cts Cursor: 10.234 (26 cts) **Energy (keV)**



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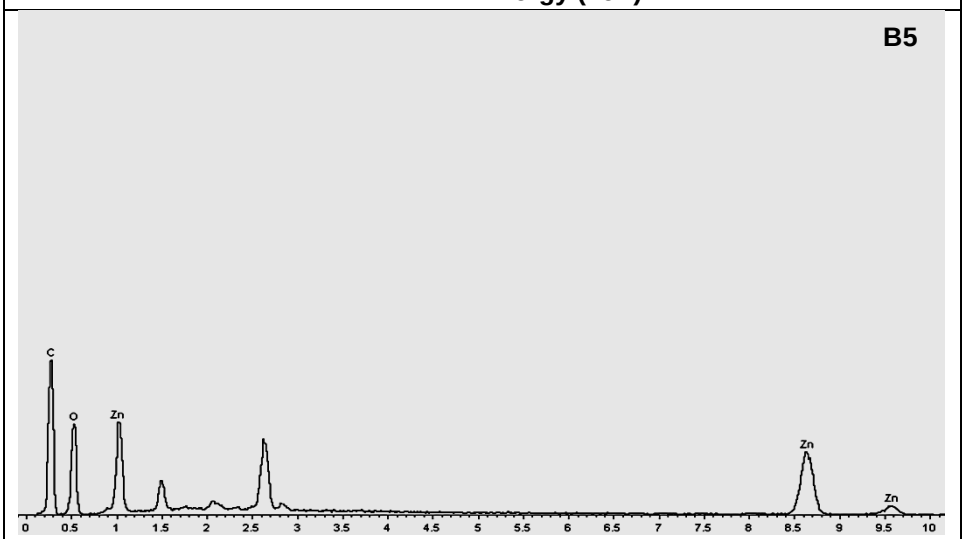


Full Scale 7667 cts Cursor: 10.234 (15 cts) **Energy (keV)**

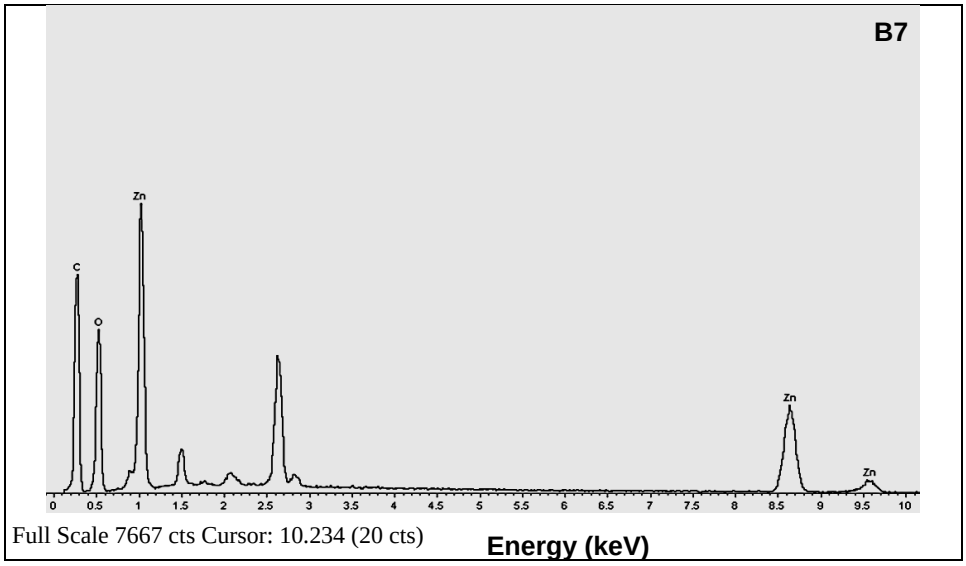


Fig. 2. SEM-EDX analysis of flocs in terms of two elements that appeared on paper surfaces

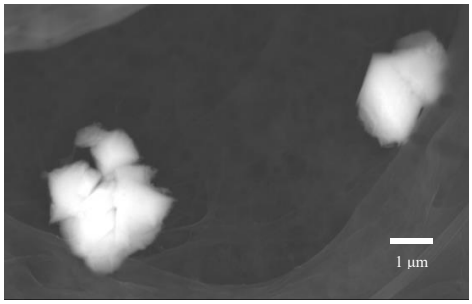


Fig. 3. Sample B3 of zinc oxide obtained in this study

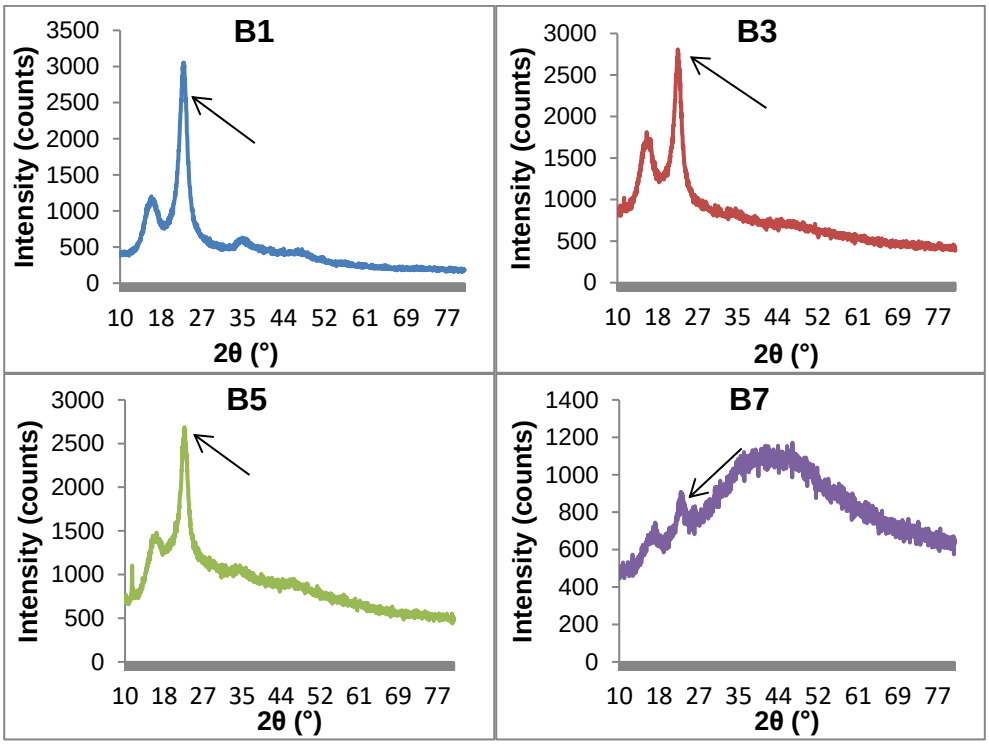


Fig. 4. The intensity peaks of crystalline structure of zinc oxide particles (shown by arrows) of samples B1, B3, B5, and B7 from XRD analysis

Chemical Composition of Zinc Oxide Pulp

The zinc oxide pulp samples were dried in sheet form and were tested for their properties in terms of the crystalline structure of zinc oxide particles by using XRD. In Fig. 4, sharp and narrow diffraction peaks of zinc oxide that attached on pulp were observed within the 2θ range from 10.00° to 30.00°, which indicated high crystallinity of zinc oxide for samples B1, B3, and B5, as achieved by Prasad *et al*. (2010). The crystallinity of prepared zinc oxide pulp decreased as the concentration of zinc chloride increased. These were presented by sharp peaks observed at 22° of 2θ. Meanwhile, sample B7 only displayed a small peak at 22.2°, indicating a reduction of zinc oxide due to a decrease of degree of crystallinity (Ramesan *et al*. 2019). In addition, a broader diffraction pattern exhibited by sample B7 indicated the amorphous structure of zinc oxide was formed (Ramesan *et al*. 2018). This is also parallel to findings by Vijayakumar *et al.* (2018) and Chikkanna *et al.* (2019) who obtained green synthesized zinc oxide that is recorded at range $20°<2\theta>80°$.

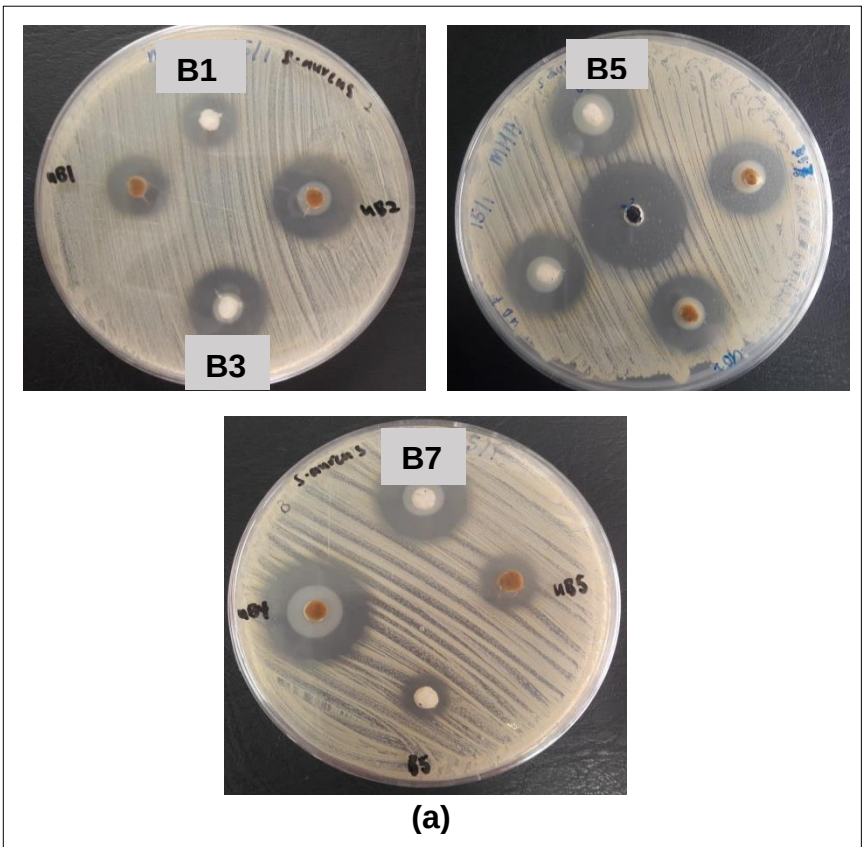
Antibacterial Test

Referring to Table 3, all samples exhibited inhibition zones from 10.00 to 25.00 mm. All zinc oxide pulp samples showed inhibition zones with increasing diameter as the concentration of the precursors increased. The average inhibition zone for pulp samples increased from sample B1 until B7 by 35% against *S. aureus*, 59% against *S. choleraesuis*, and 58% against *E. coli*. Sample B1 exhibited stronger antibacterial activity against *S. aureus*, while sample B3 successfully inhibited both *S. aureus* and *S. choleraesuis*. Meanwhile, samples B5 and B7 exhibited stronger antibacterial activities against *S. choleraesuis* with more than 20 mm of inhibition zone. Referred to findings by Alswat *et al.* (2016), parameter of concentration, increasing amount and decreasing particle size of zinc oxide contributed to inhibition zones. Interestingly, Gudkov *et al.* (2021) stated that modifying the synthesis method is one of the keys to increase antimicrobial effectiveness instead of the conventional method, namely as physicochemical.

According to Sidiqi *et al*. (2018), the size of zinc oxide nanoparticles tested against *E. coli* and *S. aureus* in the range from 401 nm to 1.2 μm showed an increasing of antimicrobial activity as the size of particles decreased. The presence of zinc oxide particles as an antibacterial agent damaged the respiratory enzymes and cytoplasmic contents in the bacterial membrane, that resulted in the death of bacterial cells (Gunalan *et al.* 2012; Vijayakumar *et al*. 2018). The penetration properties of zinc oxide nanoparticles can be explained by reaction of the oxygen released on the zinc oxide surface with hydrogen ion to produce hydrogen peroxide that can penetrate and kill the bacteria (Fang *et al*. 2006; Gunalan *et al.* 2012). According to Ohira *et al*. (2008), the smaller size of zinc oxide enhanced the production of hydrogen peroxide that strongly depends on surface area of zinc oxide. Figure 5 shows the inhibition zones for B1, B3, B5, and B7 pulp samples against *S. aureus*, *S. choleraesuis*, and *E. coli*.

Table 3. Antibacterial Screening Test Results

Sample	Inhibition Zone (mm)		
	S. aureus	***S. choleraesuis***	***E. coli***
B1	14.00	10.33	10.00
B3	18.33	18.33	16.33
B5	19.67	21.33	20.00
B7	21.67	25.00	23.67


B1
B5
B3
B7
(a)
B1
B5
B3
B7
(b)

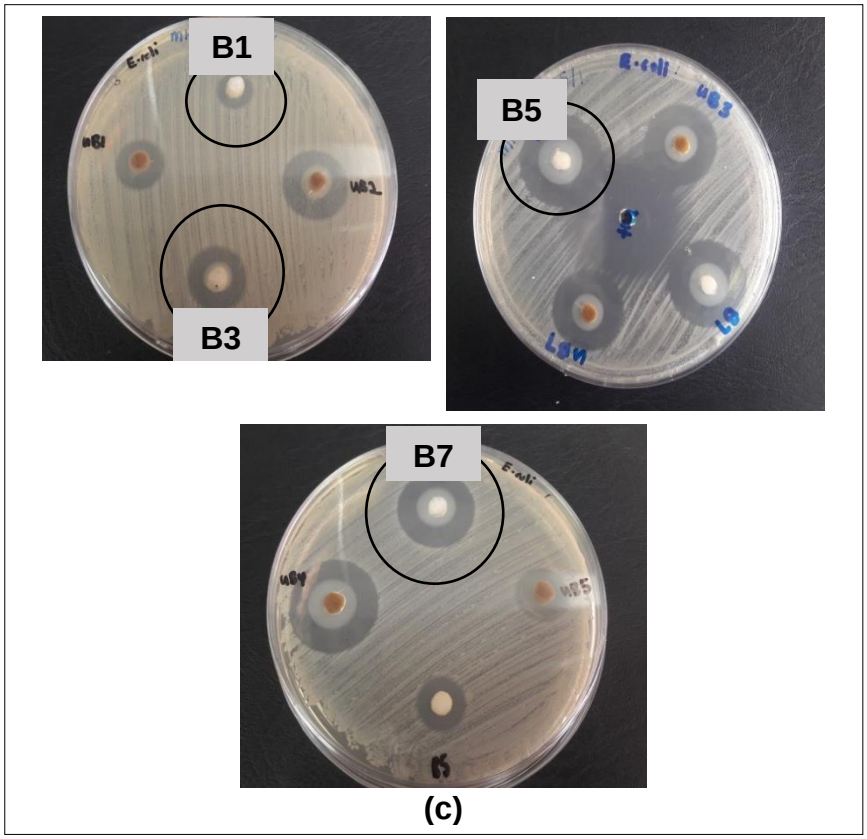


Fig. 5. Inhibition zone for samples B1, B3, B5, and B7 (black circles) against (a) *S. aureus*, (b) *S. choleraesuis* and (c) *E. coli*

CONCLUSIONS

Zinc oxide-containing paper was successfully produced *via in situ* approach, and the study can be concluded as follows:

1. Morphological observation showed that the size of the zinc oxide particles ranged from 400 to 570 nm with spherical shape. The percentages of element composition of the paper were 15.08% to 34.08% of zinc and 17.45% to 32.59% of oxygen.
2. The crystallinity of zinc oxide-pulp, prepared by a bio-mediated precipitation, decreased as the concentration of zinc chloride increased. The higher percentage of precursors developed more amorphous structure, as shown by B7.
3. Antibacterial activities against *S. aureus*, *S. choleraesuis*, and *E. coli* has shown acceptable inhibition regarding the increasing concentration of zinc chloride. It was found that more than a 30% increment of inhibition zone occurred with a diameter ranging from 10.00 to 25.00 mm.
4. Overall, the addition of precursors more than 0.3 M had higher potential to enhance the growth of zinc oxide *via in situ* preparation, hence providing better antibacterial resistance properties.

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