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The effect of natural Zeolite (Ag-Zeolite) modified with silver against the inhibition of *Candida albicans*

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ABSTRACT *Candida albicans* is a commensal microorganism or normal flora in the mouth, and about 20-75% is found in the general population without causing symptoms (Candida carriers). *C. albicans* is a primer agent that cause oral candidiasis. Silver modified natural Zeolite has potential antifungals. This research aimed to know the effect of silver modified natural Zeolite (Ag-Zeolite) on the *C. albicans* growth. Antifungals were treated through a good diffusion test. Antifungals agents were divided into four groups. They were 30 mg with 0.025 M (AgNO₃), 0.05 M (AgNO₃), 0.1 M (AgNO₃) modified natural zeolite, and unmodified natural zeolite. Each group has treated into four times well diffusion tests. This research showed that 0.1 M (AgNO₃) modified natural Zeolite has the highest inhibition. ANOVA test showed the average diameter of inhibition was significant in 0,004 (p<0,05). Each group has different average diameter inhibition. In conclusion, the active natural Zeolite with silver modification can inhibit the growth of *C. albicans* better than natural Zeolite without silver modification.

KEYWORDS: *C. albicans*, natural Zeolite, silver

INTRODUCTION

Many domestic medicinal raw materials are still imported, so this impacts the high price of pharmaceutical products in Indonesia. Indonesia's geographic area supports the availability of mineral materials that can be used as medicinal raw materials, such as areas with high rock content. Zeolite is a natural mineral that is abundant around volcanoes.¹ Indonesia has a mountain range from Sumatra, Java, Bali, Nusa Tenggara, Sulawesi to Papua. Natural Zeolite is a biocompatible coating containing a crystal structure and is easy to modify with metals with antifungal properties.²

There are 20 locations in Indonesia with 447,490,160 tons of zeolite deposits. Place, West Java Province, has the highest zeolite deposit resources of 185,595,160 tons.³ Natural zeolite in Indonesia consists of mordenite and clinoptilolite types. It levels vary and are usually mixed with impurity oxides of the type of silica and iron compounds. In addition to dissolving impurity oxides, the HF refining process can also remove alkaline earth

metals and dissolve some of the framework's aluminum.⁴

The antifungal agent can be obtained by modification of natural Zeolite with silver metal. Silver ion and silver-based materials have been known to have high toxicity to microorganisms, and among them are the 16 major species of bacteria. Silver nanoparticles can cause cells to lysis and inhibit the growth of microorganisms.⁵ Silver in Zeolite can inhibit *C. albicans* and release 0.86 ppm silver in water for 24 hours from a 2% mass fraction of silver zeolite cement sample.⁶

Oral candidiasis is an opportunistic infection in the oral cavity. *Candida* is a commensal microorganism or normal flora in the mouth, and as much as 20-75% are found in the general population without causing symptoms (Candida carriers). The *C. albicans* is the primary causative agent for oral candidiasis. It has predominantly resided on the tongue posteriorly's dorsum. About 57.1% of people with candidiasis complained of pain, burning, or

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burning, and about 4.1% complained of a burning/ burning sensation during activities, especially when eating and drinking. It caused *Candida* spp attached to the oral cavity's mucosal surface or tongue through its hyphae can be detached and leave red mucosa and sometimes light bleeding dan

cause pain and burning in the oral cavity as a sign of inflammation.⁷ This study aims to determine the effect of natural zeolite modification with a silver (Ag-Zeolite) on *C. albicans* fungi's growth.

3 MATERIALS AND METHODS

This research is experimental laboratory research with a post-test only control group design or after only control group design. Activation and modification of natural zeolites were carried out in the Quantitative Chemistry Laboratory of Bhakti Wiyata Kediri. Characterization test for Zeolite and silver modification was carried out in the Mechanical Engineering laboratory of the FTRS Institute of Technology Surabaya. Meanwhile, the antibacterial activity test was carried out in the Bhakti Wiyata Institute of Health Sciences' Bacteriology laboratory.

Natural Zeolite Preparation, Activation, and Ag-Zeolite Modification

Natural Zeolite (PT. Asia Zeolite Prima, Surabaya Indonesia) 100 mesh size was washed with water and continued drying at a temperature of around 105°C. As much 6g zeolite powders was activated by immersing in 500mL of 3M HCl (Sigma-Aldrich) solution for 12 hours. It was filtered, washed with distilled water 16 times or until the pH was neutral, and dried using an oven at 150°C for 5 hours. Then the zeolite sample was immersed in 4M NaCl solution (PT. Smart-Lab Indonesia) for 24 hours, after which the zeolite sample was filtered and washed with distilled water 16 times or until the pH was neutral and dried using an oven at 150°C for 5 hours. The modification of Zeolite by silver was done by immersing 6g of active Zeolite in 60ml 0.025M, 0.50M, and 0.1M AgNO₃ (Merck 99.8%) stirring for 5 hours. The silver-modified Zeolite was

washed with distilled water (16 times) and dried using an oven at 150°C for 5 hours.⁸

Preparation of *Candida albicans* Suspension

Stocks of *C. albicans* were collected by sterile cotton, suspended into a tube containing 3ml BHIB (Merck, Germany), homogenized, and incubated at 37°C for 24 hours. After 24 hours, the turbidity of the test suspension was equated with the Mc. Farland standard 0.5 (1,5x10⁸). A solution is prepared by mixing 0.5ml of barium chloride with 99.5ml of sulfuric acid until a homogeneous solution is obtained. The turbidity of *C. albicans* in the liquid medium was compared with the turbidity of the standard.

Inhibition Assay

SDA media (Merck, Germany) has been swabbed with *C. albicans* cultures until evenly distributed and left for 5 minutes, and this is done to culture *C. albicans* absorbed on the media. The well filled with silver modified Zeolite 0.025M, 0.50M, and 0.1M AgNO₃ and a zeolite control group without silver modification. Each concentration was added to the well 30 mg and added with 50ul of aquadest. SDA media planted with *C. albicans* and zeolite test samples were incubated at 37°C for 24 hours. After 24 hours, the inhibition was observed by measuring the inhibition zone's diameter with a glass loop and a caliper. The data obtained from the research group were then analyzed using the ANOVA test.

RESULTS

The zeolite surface has been subjected to morphological characterization by SEM analysis (Hitachi Flexsem 100). Based on Figure 1A, the natural zeolite surface that has not been activated still has an image of the Zeolite that has not opened its pores. Based on Figure 1B, the surface of natural Zeolite that has been activated has an image of

available Zeolite pores because the impurity material in natural Zeolite has begun to dissolve during washing. After the active zeolite surface is given silver modification, Figure 1C can see an image of the material that closes the zeolite surface's pores.

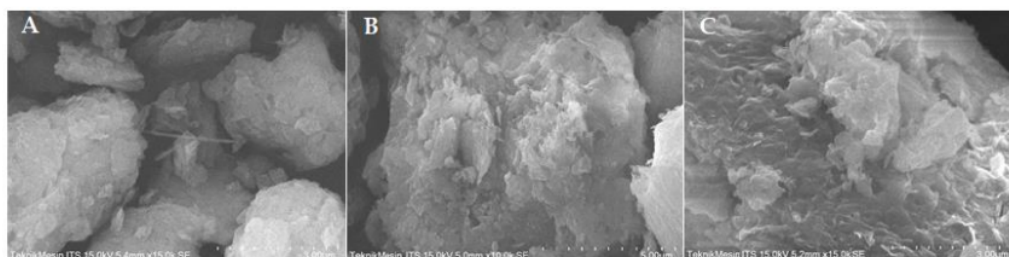


Figure 1. The SEM morphology of Zeolite. (A) Natural zeolite surface morphology prior to activation, (B) Surface morphology of natural zeolites after activation with 3M HCL + 4M NaCl (C) active zeolite surface morphology after silver modification. Magnification X15000.

The zeolite component elements (before activation, after activation, and which have been modified silver) have been tested for EDS analysis (Hitachi Flexsem 100). Based on **Table 1**, there are differences in the amount of silver element from

zeolites that have been modified with different $AgNO_3$ concentrations. Apart from the silver piece, several mineral elements decrease and increase during the zeolite activation process.

Table 1. EDS tested the composition of active Zeolite before and after modification of silver (Ag)

Zeolite	Types of minerals (%)							
	Na	Mg	Al	Si	K	Ca	Fe	Ag
Zeolite before activation	0,9	0,89	8,34	33,75	2,63	2,63	1,77	0
Zeolite active	1,81	0,63	6,15	39,39	1,06	1,07	1,15	0
Zeolite aktifewith $AgNO_3$ 0,025 M	1,25	6,47	0	38,93	0,94	0	0	2,28
Zeolite active with $AgNO_3$ 0,5 M	0,85	0,65	6,23	39,29	1,14	0	0	4,15
Zeolite active with $AgNO_3$ 0,1 M	0,64	0,66	5,94	38,53	1,02	0	0	5,74

Based on **Table 2**, the inhibition zone's mean diameter shows the area of *C. albicans* that did not grow on SDA media. The larger the inhibition zone, the larger the area of *C. albicans* that does not increase. The ANOVA test results showed that the

inhibition zone's significance value was 0.004 ($p < 0.05$), which means a significant difference in the study group. It has been assumed that the modification of natural Zeolite with silver affected the growth of *C. albicans* microbes.

Table 2. The average diameter of *C. albicans* inhibition zone after 24 hours of incubation times

No	Sample Zeolite	Average (mm)
1	Active Zeolite Without silver	10
2	Active Zeolite + Silver 0,025 M	12,5
3	Active Zeolite + Silver 0,05 M	16,5
4	Active Zeolite + Silver 0,1 M	20,75

DISCUSSION

The chemical activation process is carried out by adding hydrochloric acid (HCl) and then washing it using distilled water until pH neutral. Reducing natural zeolite particles' size to 100 mesh aims to expand the surface, increasing natural zeolites' activity. The addition of acid results in cations with H^+ , thereby enlarging the zeolite framework's cavity and increasing its adsorption power. The metal content of Ca and Mg has

decreased. The ion exchange is influenced by the cations from the Zeolite and the protons from HCl.⁹ The effect of activation of the acid treatment causes dealumination and decationation, namely the release of alumina and cations (Mn^+) in the framework. Zeolite can be crystallized by acid treatment using HCl. The dealumination process will increase the Zeolite's surface area due to reducing impurity metals covering the zeolite pores.

Inhibition test of silver-modified Zeolite against *C. albicans* microbes' growth has shown that natural Zeolite and silver have antifungal properties. Antifungal materials can be of organic and inorganic origin. The organic antifungal material is mostly triclosan and benzalkonium chloride, while the inorganic antifungal material is derived from heavy metals.² Inorganic antimicrobial materials are more stable than organic antifungal materials because organic materials have more volatile properties than inorganic ones.

The *C. albicans* caused opportunistic infections in humans by forming a biofilm film. Extracellular DNA (eDNA) in *C. albicans* plays a role in forming biofilm layers and biofilm structure.¹⁰ The biofilm layer has a matrix structure that can determine its stability in the host cell. The attachment to the host cell serves as a source of nutrition for the fungus. The ability to form a biofilm layer is a significant factor in the virulence of *C. albicans*.¹¹

Silver nanoparticles can bind to fungal cell walls and continue penetration into fungal cells, resulting in structural changes in fungal cells. Free radicals from silver nanoparticles can damage cell membranes and make cell membranes porous.¹² Cell membranes damaged or passable will make it easier for silver nanoparticles to react with these cell bases. Cell DNA has the main components, namely sulfur and phosphorus, as the base of the cell. The interaction between silver nanoparticles and DNA

will result in fungal DNA replication problems, resulting in fungal death.¹³

Synthetic Zeolite (NaY zeolite, CBV100), which is a product of the Zeolyst International-USA company which has been modified with 0.01M, 0.025M, and 0.05M AgNO₃ and followed by modification of 0.05M Zn(NO₃)₂ with a ratio (20 mL of solution/gr zeolite) indicates antifungal power.¹⁴ Concentrations of 0.01 to 0.10 mg / mL (Zn0.05Ag0.025-Y) are the most efficient concentrations in inhibiting fungi and bacteria. There is an attraction between the positive charge of the modified zeolite material (Ag⁺ and Zn²⁺) and the negative charge from the microbial cell membrane to interfere with the membrane's permeability antimicrobial particles can penetrate the cell.¹⁵

Synthetic Zeolite with the ZSM-5 brand is an active zeolite that does not contain organic substances and has a high SiO₂/Al₂O₃ modified ratio with 0.1N AgNO₃, which is proven to have antifungal properties. The *C. albicans* exposed to ZSM-5 (Ag) for 24 hours at an incubation temperature of 36 ° C show an inhibition zone diameter of 13mm (10mm for zeolite diameter and 3mm clear zone around Zeolite).¹⁶ This research shows that Indonesian natural zeolites have the same potential antimicrobial carrier materials as zeolites from foreign products. It requires better development efforts to explore the potential of Indonesian natural zeolites antimicrobial carrier materials.

CONCLUSION

The active natural Zeolite with silver modification can inhibit the *C. albicans* better than natural Zeolite without silver modification.

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