

PRELIMINARY STUDIES ON ANTIFUNGAL AND ANTIBACTERIAL ACTIVITY OF ALUM AS MEDICINE PREPARATION FOR VAGINAL DISCHARGE



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ABSTRACT

Background: Alum has many functions in various fields such as an astringent substance has the potential to inhibit the growth of microorganisms. This study aims to know the antifungal and antibacterial activity against *C.albicans* and *N.gonorrhoeae* which can be used as medicine preparation for vaginal discharge. These microbes are pathogenic microorganisms commonly found in the vagina.

Method: The method used is the disk diffusion which is based on a clear zone area to determine the inhibition. Variation concentration of alum used is 2.5; 5; 10; 15; 20 and 25%.

Results: The results showed that alum has antimicrobial activity against pathogenic microbes, namely *C.albicans* and *N.gonorrhoeae*. This is indicated by the presence of clear zones formed in the area of paper disks. Concentrations of 20% and 25% have moderate antimicrobial activity. An increase in concentration in alum shows that the diameter of the clear zone that is formed is also getting bigger.

Conclusions: In this study, it can be concluded that alum is proven to have antifungal and antibacterial activity against pathogenic microbes that live in the vagina. So, it has the potential to become one of the medicine compositions for vaginal discharge.

1. INTRODUCTION

Potash Alum is an inorganic salt crystalline formed with double sulfate which is generally odorless, colorless, and soluble in water. Alum can be used as astringents and antiseptics in various food preparation processes such as preservation and fermentation, and also for water purification as flocculants [1]. The molecular formula of alum is $KAl(SO_4)_2 \cdot 12H_2O$, if the solution of potassium aluminate sulfate dodecahydrate which is almost saturated is cooled, it will form crystals octahedrons. Aluminum sulfate salt solution is acidic meaning that hydrolysis of this salt produces precipitated $Al(OH)_3$ and ions H_3O^+ which carry acidic properties [2]. The form that is often found is a form of Hexa decahydrate and octa decahydrate. Whereas natural minerals have the same empirical formula as

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dehydrate. Alum solution is acidic if tested used litmus paper. Alum is very soluble in boiling water and is easily soluble in water, insoluble in alcohol, and soluble in glycerine [3].

Alum can be used as astringents where are substances that cause contractions or shrinkage of tissues and dry secretions. It can also be used as a post-shaving treatment, can eliminate bleeding from superficial wounds. Alum also showed antimicrobial activity in vitro tests [4]. According to (Drugbank) Potassium alum is considered by the FDA as a substance that is generally known as a safe substance, this compound is an inorganic salt [5]. In Bnyan research showed that Aluminum potassium sulfate (alum) has the ability of antibacterial activity and inhibits the growth of gram-positive and gram-negative bacteria isolated from various infection sites [6].

Vaginal discharge is a condition that is often experienced by women throughout their life cycle. In a normal (physiological) or abnormal (pathological) state as a sign of disease [7]. In women who experience abnormal vaginal discharge can be an indication of various diseases such as vaginitis, candidiasis, and trichomoniasis. Bacterial vaginal discharge (BV) is the most common vaginal abnormality in infertile women [8]. BV can cause vaginal discharge or odor. *Candida albicans* and *Neisseria gonorrhoeae* are pathogenic microorganisms classified as BV. Its existence can make pathological vaginal discharge that can lead to sexual diseases. Diseases caused by candida are called candidiasis, whereas diseases caused by *Neisseria gonorrhoeae* are called gonococci [9].

Information about alum used as a medicine for vaginal discharge is still limited. While the community has used it as a natural remedy used to treat vaginal discharge because that alum has a function as astringents substances. Generally, alum is used for the water purification process, and in the food sector, it is often used to improve the food sector. To strengthen this, a preliminary study is needed to know antifungal activity against *Candida Albicans* and antibacterial against *Neisseria gonorrhoeae*. So, this research was conducted to strengthen the use of alum as medicine preparation for vaginal discharge.

2. MATERIALS AND METHODS

2.1. MATERIALS

Potassium aluminium sulphate (Alum) $KAl(SO_4)_2 \cdot 12H_2O$, *Candida albicans* ATCC10231, *Neisseria gonorrhoeae* 49226, Ketokonazol 2%, Ciprofloxacin 5 μ cg, Sabouraud Dextrose Broth/SDB (Sigma-Aldrich), Potato Dextrose Agar/PDA (Merck Germany), Thayer-Martin medium, blank disk and sterile Aquades, tissue.

2.2. METHODS

2.2.1. SAMPLE PREPARATION

Alum in the form of crystals pounded using mortar and pestle. Then weighed using an analytical balance at as much as 0.25 g; 0.5 g; 1 g; 1.5 g; 2 g; and 2.5 g. Then dissolve it with 10 mL of distilled water and then filter it using filter paper. So that the concentration obtained in percent is 2.5%; 5%; 10%; 15%, 20% and 25%. The sample solution is ready to be tested on antifungal and antibacterial activity with the disk diffusion method.

2.2.2. ANTIFUNGAL TEST

To determine antifungal activity carried out by the disk diffusion method, there are 2 media used, namely SDB (Saboroud Dextrose Broth) and SDA (Saboroud Dextrose Agar). SDB was put into a test tube and then taken 1 osse of the candida *Albicans* and then incubated at 37° C for 24 hours. After that, the SDA media is poured into each disposable Petri amount of concentration variation. Using the streaking method and each fungal colony was swabbing were carried out on the SDA media. Sterile disk paper saturated alum samples with a concentration variation of 2.5%; 5%; 10%; 15%, 20% and 25% for 15 minutes, then planted on SDA media that already contain candida using tweezers and slightly pressed. The positive control using 2% Ketoconazole [10]. And the negative control using distilled water (aqua dest) because the solvent is used to dissolve alum samples using distilled water. Then incubated for 24 hours at 37°C. After that, it was observed the presence or absence of a clear zone area around

the paper disk and measured using calipers millimetres (mm). The clear zone area formed shows that there is an antifungal activity [11].

2.2.3. ANTIBACTERIAL TEST

Neisseria Gonorrhoea suspension with 1 mc Farland turbidity then put the sterile swab into the germ suspension. After that, sing the streaking method evenly onto the Thayer-Martin media. Sterile blank disks are immersed or saturated with a sample of alum solution with variation concentrations of 2.5%; 5%; 10%; 15%, 20% and 25% for 15 minutes. Then the disk that has been soaked is planted on the Thayer-Martin media. A positive control using ciprofloxacin 5 mcg antibiotic and negative control using distilled water because the solvent is used to dissolve alum samples using distilled water. All Petri dishes were incubated at 35 - 37°C for 24 hours. After that, observed the presence or absence of a clear zone area around the paper disk and measured using calipers in millimetres (mm). The clear zone area formed indicates that there is antibacterial activity.

3. RESULTS AND DISCUSSIONS

The results obtained in this study are shown in Table 1 and Table 2. In both antimicrobial activity assay (antifungal against *Candida albicans* and antibacterial against *Neisseria gonorrhoeae*) is determined by the presence of clear zones in the paper disk area formed (shown in figure 1) that the inhibition zone diameter values are obtained. The study of Akkaoui said that antimicrobial activity is assumed if the zone area formed is greater than 6 mm if the disk paper is included in the calculated of the determination of the inhibitory zone [12].

Based on the data, if the diameter of the disk paper is included in the calculation then the alum samples that have been tested have antimicrobial activity. Inhibition zones that formed on the growth of *Candida albicans* began to be seen at a concentration of 10%. Whereas the two lowest concentrations of 2.5% and 5% have no clear zone area. In alum antibacterial assay against *Neisseria gonorrhoeae* showed to have antibacterial activity. This is indicated by the formation of a clear zone area in the area of paper disks at all concentrations of 2.5%; 5%; 10%; 15%, 20%, and 25% have antibacterial activity.

Table 1: Results of measurement of inhibition zone diameter (mm) of antifungal assay on alum against the growth of *Candida Albicans*

Alum Concentration (%)	Inhibition Zone Diameter (mm)				Average IZ (mm)
	I	II	III	IV	
2,5 %	0	0	0	0	0
5%	0	0	0	0	0
10%	4.85	3.3	4.3	3.1	3.89
15%	4.3	4.65	4.15	4.8	4.48
20%	5.7	6.1	5.35	5.3	5.61
25%	6	6.1	6.35	6.5	6.24
Control (+)	7.5	10.25	9.65	7.1	8.63
Control (-)	0	0	0	0	0

Table 2: Results of measurement of inhibition zone diameter (mm) of the antibacterial assay on alum against the growth of *Neisseria Gonorrhoeae*

Alum Concentration (%)	Inhibition Zone Diameter (mm)				Average IZ (mm)
	I	II	III	IV	
2,5 %	2	2	2	2	2
5%	2	3	3	3	2.75
10%	4	4	4	3	3.75
15%	5	5	4	4	4.5
20%	9	9	13	6	9.25
25%	12	9	9	9	9.75

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Control (+)	43	43	43	43	43
Control (-)	0	0	0	0	0

Antifungal and antibacterial in this study aim to know the Antimicrobial activity of alum against pathogenic microbes that live in the vagina cause abnormal vaginal discharge. Abnormal vaginal discharge (pathological) is usually yellow, grayish-green, fishy, foul-smelling and the amount of vaginal fluid in large amounts can cause complaints of itching and burn in the intimate area [13]. The main cause of the abnormal vaginal discharge is a vaginal or cervical infection, both of which are caused by the presence of harmful microorganisms such as germs, fungi, viruses and parasites, and tumors [14].

Antimicrobials are substances that can kill or inhibit the growth of other microorganisms [15]. *Candida albicans* species is a normal flora that normally lives in the digestive tract and mucous membranes, respiratory tract, vagina, urethra, skin and are carried by the fingers and toenails [16]. This infection caused by a fungus is called candidiasis. In vulvovaginal candidiasis usually causes complaints of itching, dyspareunia, vaginal discharge, reddening in the vagina, sometimes pain when having sex or urinating, swelling of the vulva and labia with discrete pustules papules lesions, and usually symptoms worsen before menstruation [17].

While gonorrhoea is an infection caused by *Neisseria gonorrhoeae*, which is an aerobic gram-negative diplococci bacteria. In this disease commonly referred to as gonococcus, the fluid that comes out of the vagina is yellowish, which is pus consists of white blood cells that contain bacteria *Neisseria gonorrhoeae* in the form of twos in the cytoplasm of cells.

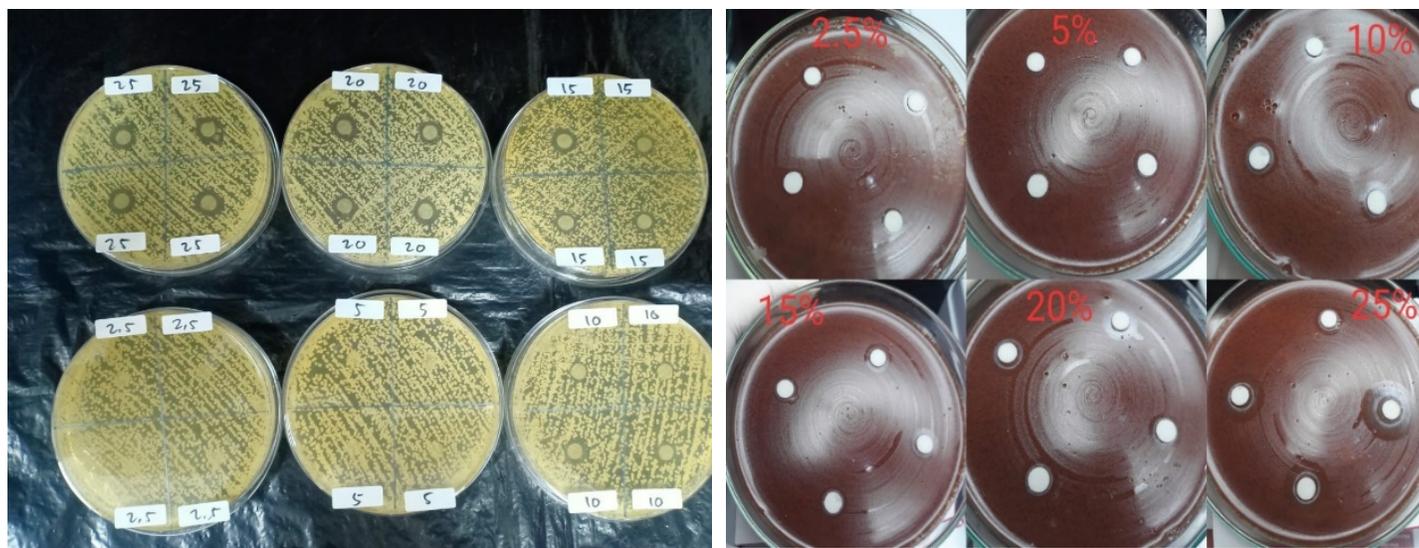


Figure 1: Inhibition zone diameter of alum against on the growth of *C. albicans* (left) and *N. gonorrhoeae* (right) with various concentrations of 2.5; 5; 10; 15; 20; and 25%.

The mechanism of inhibition of bacterial growth by antimicrobial compounds can be in the form of cell wall damage. The damage can also be in the form of changes in the permeability of the cytoplasmic membrane, causing food to escape from the cell. Antibacterial compounds can work bacteriostatic, bactericidal, and bacteriolytic [18]. Besides that salt also has fungicidal properties that can work effectively against gram-positive and gram-negative bacteria [19].

Potassium alum (potash alum) is formed by large transparent crystals that are used in various products such as food or medicine as buffers, neutralizers, or forming agents. Its use in society is very diverse including cosmetics or astringents, helping to shrink tissue, formulations in cleaning products, antiperspirants, and antifungals. Its properties are almost the same as salt can function as a barrier to the growth of pathogenic decomposing microorganisms because it has antimicrobial properties [20]. The main function of alum as astringent substances (properties that can reduce pH and shrink the tissue so that it can inhibit the growth of pathogenic bacteria). Alum can reduce the water content of microorganisms and bind it in its use in the presence of osmotic pressure so that the

cell will lose water and become dry due to shrinking contraction. Alum is a double salt hydrated made from water molecules and two kinds of salt, one of them $(Al_2(SO_4)_3)$

In this study, alum is used to antifungal assay and antibacterial against microbial pathogen *Candida albicans* and *Neisseria gonorrhoea* showed antimicrobial activity with the formation of a clear zone area around the disk as shown in figure 1. Previous studies conducted by Bryan stated that aluminum potassium sulfate (alum) has the ability of antibacterial activity and inhibits the growth of gram-positive and negative bacteria isolated from various places Gram-negative bacteria, one of which is *Klebsiella pneumonia*, which can cause urinary tract infections and bacteremia with lesions in weak patients [6]. The results show that 20% of alum is considered as MIC (Minimum Inhibitory Concentration) and the inhibition of bacterial growth increases when alum concentration also increased.

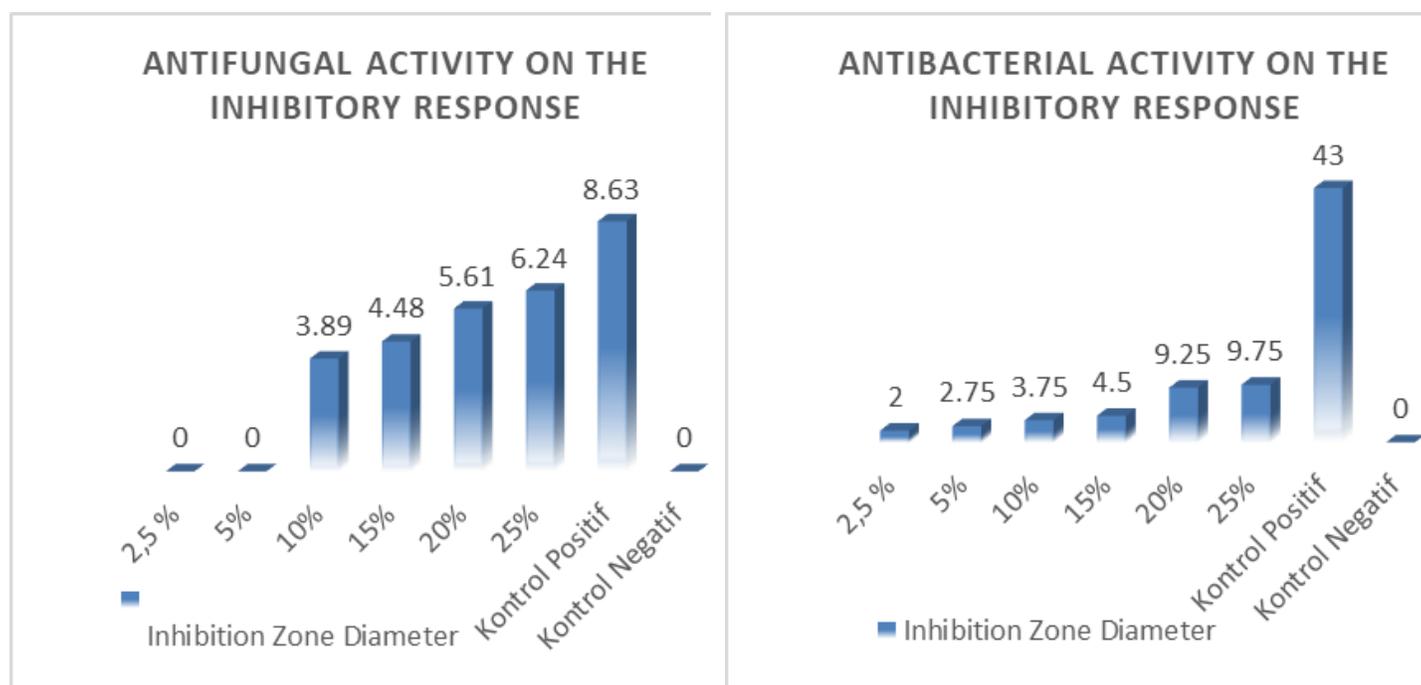


Figure 2: Graph of measurement of inhibition zone diameter of in alum on the growth of *Candida albicans* with positive control of Ketoconazole 2% (left) and *Neisseria gonorrhoeae* with positive control ciprofloxacin 5mcg (right) with a concentration of 2.5; 5; 10; 15; 20; 25% and negative control aqua dest

This study shows an increase in the value of the clear zone diameter in each concentration variation. Increase the inhibition zone diameter at each concentration of 2.5; 5; 10; 15; 20; and 25% (see figure 2). According to Davis and Stout in Rahmi's research said that the effectiveness of microbial growth inhibition responses is classified into 4 groups: Weak (diameter ≤ 5 mm), moderate (diameter 5-10 mm), strong (diameter 10-20 mm), and very strong (diameter ≥ 20 mm) [21]. Based on the data shown in table 1 for the antifungal the largest average diameter results are alum with a concentration of 25%. When compared with positive control the ratio is not too large, because at a concentration of 25% (6.24 mm) and positive control (8.63) its effectiveness is moderate. Likewise, a concentration of 20%, the antifungal activity is moderate, and the value of 5.61 mm. At concentrations of 10% and 15% have weak antifungal activity with values of 3.1 mm and 4.48 mm. Whereas the two lowest concentrations do not have antifungal activity. This is due to the formation of no clear zone area around the disk at both concentrations.

The response of inhibition of bacterial growth to *Neisseria gonorrhoeae* in alum with concentrations of 20% and 25% is moderate, but when seen in table 2 the value is almost toward the strong categories namely 9.25 mm and 9.75 mm. The results obtained have a large enough compared with the resulting positive control value that is equal to 43 mm. However, antimicrobial activity of alum are still more effective in inhibition of gonorrhoea bacteria than candida. The results are almost close to the strong category with concentrations of 20% and 25% in inhibiting the growth of gonorrhoea bacteria. (see figure 3). Alum can form flocks and bind microbes both fungi and bacteria. Although its activity is smaller than the positive control, alum can lock in the movement of microbes in the herd, so that it has no potential to spread.

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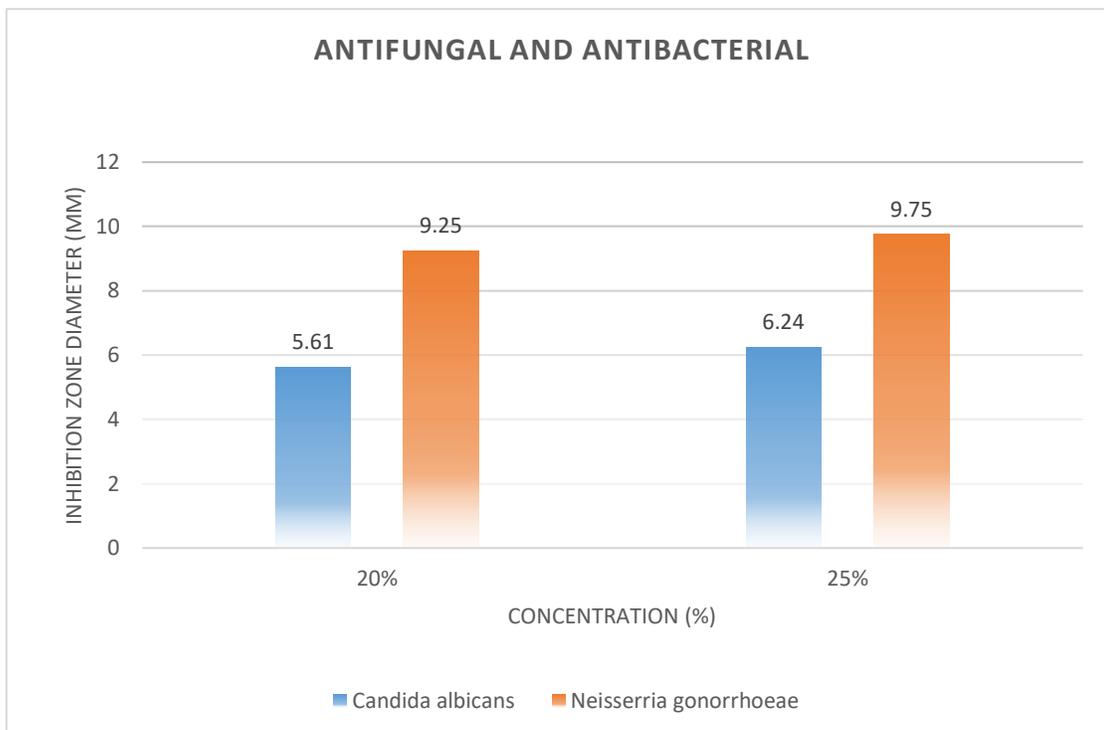


Figure 3: Comparison graph between antifungal assay (*Candida albicans*) and antibacterial assay (*Neisseria gonorrhoeae*) at concentrations of 20% and 25%

Ali's research said that some assumptions linked the antibacterial effects of alum with a reduction in acidity or a damaging effect on bacterial cell walls [22]. Also, histological studies prove the safety of alum salts for mammalian consumption [23]. In the Unani medical system, Tawas is a very useful and valuable drug that is used in various types of diseases such as antiseptic, antipyretic, hemostyptic, resolvent, etc. [24].

In this preliminary study showed the existence of antimicrobial activity in alum to inhibit pathogenic microbes that normally live in the vagina, namely the fungus *Candida albicans* and the bacterium *Neisseria gonorrhoeae*. The response of the inhibition to the test microbes is indicated by the formation of a clear zone in the disk area. The diameter value formed indicates that alum can inhibit pathogenic microbes and has the potential to become one of the vaginal discharge medicine compositions. Its ability to form flocks to bind microbes is very beneficial for herbal medicines by vaginal use or by oral.

4. CONCLUSIONS AND RECOMMENDATIONS

Alum has antimicrobial activity against the *C. albicans* and *N. gonorrhoeae* which is indicated by the formation of a clear zone area around the disk. Antifungal and antibacterial activities at concentrations of 20% and 25% are in the moderate category. However, the inhibition response to *N. gonorrhoeae* has an ability that is almost to the strong category when compared with antifungal against *C. albicans*. Alum has the potential to be one of the medicine compositions for Vaginal discharge.

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CONFLICT OF INTEREST

None.

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