Formulation of Liquid Soap Ethanol Extract from Batak Onion as Antifungal against Candida albicans

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Keywords: Liquid soap, Antifungal, Batak onion, Candida albicans.

Abstract: Batak onions (Allium chinense) grow widely in North Sumatra, Indonesia. The onion is one of the natural ingredients that has the potential as an antifungal due to the content of active compounds such as saponins, flavonoids, triterpenoids and steroids. This research is aimed to look at the antifungal effects of Candida albicans ethanol extract on batak onions after it is formulated into liquid soap by varying the concentration of 1%, 5% and 10%. Liquid soap is made using a base of sodium lauryl sulfate soap, citric acid, sodium chloride, propylenglycol and water. Batak onion extract by maceration method for 5 days using 96% ethanol solvent while stirring occasionally and then filtered. Antifungal power test used the fungus Candida albicans in PDA medium. Evaluation of batak onion extract liquid soap includes organoleptic examination, pH test, macroscopic and microscopic examination and antifungal effect test. The results showed that the concentration of ethanol extract of 1%, 5% and 10% onion had antifungal activity against Candida albican.

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1 INTRODUCTION

Candida albicans is a species of fungus found in several parts of the body of healthy organs, such as in the mouth, throat, intestine, genital tract, faeces, under the nails and skin Candida mushroom cells are oval in shape or oval in their colonies on a dense medium slightly arising from the surface of the medium with a smooth, smooth or folded surface, yellowish white and yeasty. Large colonies depend on age. At the edge of the colony all hyphae can be seen as fine threads belonging to the medium. In liquid medium the fungus usually grows on the bottom of the tube (Suprihatin, 2017; Bahari, 2018).

Fungus is one of the causes of infection in diseases, especially in tropical countries. Fungal skin disease is a skin disease that often arises in Indonesian society. The tropical climate with high humidity in Indonesia strongly supports the growth of fungi. The number of fungal infections is also supported by the large number of Indonesian people below the poverty line so that the problem of environmental hygiene, sanitation and healthy lifestyles is less of a concern in the daily lives of Indonesian people (Harahap, 2012).

Most infections are endogenous because the fungus is already present in the patient's body in a variety of organs, especially the intestine. Infection usually occurs when there is a predisposing factor. Therefore Candida albicans is essentially included as an opportunist fungus (Suprihatin, 2017).

Indonesia is widely known as the second largest biodiversity mega-flashlight after Brazil in the world, which consists of 7,000 types of plants that have medicinal properties. The genus Allium (onion tribe) has been widely studied because of its potential as an antibacterial and antifungal and food preservative. One of the onion plants that have been widely used by the people, especially the Batak tribe, is the Batak onion (A.chinense) (Rosdiyawati, 2018).

For the treatment of candidiasis antifungal therapy is needed. However, the use of antifungal drugs can cause resistance to fungi and can cause side effects (Setiabudi, 2017). There are other options in treating candidiasis, namely by utilizing traditional medicine. These onion plants can inhibit the growth

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of microorganisms such as fungi that cause vaginal discharge. From the results of this study it is expected that women who experience vaginal discharge can apply the Batak onion plant as a non-pharmacological drug and make the Batak onion plant a family medicinal plant. (Ginting Munthe, N., Sembiring, I., & Siregar, W., 2019).

Patients with candidiasis are usually given topical antifungal drugs that have been used to treat skin candidiasis including Nystatin, Clotrimazole, Miconazole and other Azol groups. However, antifungal drugs have limitations such as severe side effects, narrow spectrum antifungal, poor penetration in certain tissues and the emergence of fungal resistance (Setyowati, 2017). Therefore it is necessary to find other safer alternative treatments.

Batak onion (Allium chinense) is a type of plant that is also a food ingredient. The chive shape is like an onion but with the tip of a longer stem and the color tends to be white. So it resembles a shiny shaped leek with long small leaves and also looks like an onion, but its size is much smaller, but different from onion chives (Lin et al, 2016).

Allium Chinense also has antimicrobial activity due to the content of alisin and thiosulfate in it. The transformation form of the two compounds is also referred to as having quite potent anti-microbial activity. Therefore allium chinense can inhibit many microorganisms, such as fungal bacteria, viruses and parasites (Dian, et all, 2017).

Natural compounds from various Allium species that have antimicrobial activity consist of several classes of chemical compounds including saponins, flavonoids, phenols, alkaloids and peptide proteins. These onion plants can inhibit the growth of microorganisms such as fungi that cause vaginal discharge. From the results of this study it is expected that women who experience vaginal discharge can apply the Batak onion plant as a non-pharmacological drug and make the Batak onion plant a family medicinal plant. (Ginting Munthe, N., Sembiring, I., & Siregar, W., 2019).

The sulfur content of onions which mostly become sulfonates and polysulfides are important compounds to prevent cancer, heart disease, hypertension and diabetes. A study states that the organosulfur content has an influence on plasma cholesterol and atherosclerosis in vitro, treating bronchitis, pleurisy, angina pectoris, shortness of breath and diarrhea (Lin et all, 2016). One of the famous genus of plants and widely used by the community is Allium. More than 280 genera of Allium are spread throughout the world (Robinowitch, 2018).

Most of this genus is used by the community as a spice in cooking and traditional medicine. Allium is widely used as an antimicrobial and antifungal. In addition, Allium is also used in food preservation to replace chemical compounds that are widely used in the food industry. Various antimicrobial compounds of this genus have long been known as allicin, diallyl disulfide, ajoene, and 3 (allyltrisulfanyl) -2-amino-propanoic acid which has been proven to inhibit the growth of microorganisms such as bacteria, fungi, viruses and parasites. Antimicrobial compounds which were studied from Allium extract are believed to be able to help solve the problem of pathogenic microbial resistance arising from the use of antibiotics (Dian, 2017; Yasni, S, 2016).

Soap is potassium or sodium salt from fatty acids derived from animal fats and vegetable oils. Currently, liquid skin cleansing soap is the most widely used by the public because liquid soap is more practical, more economical, easy to carry, more hygienic for shared use, and easy to store. Liquid soap is effective for removing impurities that stick to the surface of the skin both water soluble or fat soluble (Rosdiyawati, 2018).

The using of a base of liquid soap can affect the effectiveness of the coir. Liquid soap base that gives good results, namely olive oil and potassium hydroside (KOH) because it provides the effect of reducing dry skin after 35 days of use and does not cause irritation to the skin. Besides soap can be used to treat diseases, such as skin diseases caused by bacteria and fungi by cleaning the body and the environment so that the possibility of disease and infection will be reduced (Agus, P., Ali M., 2017).

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Batak or chive is an onion plant that has often been used by the Batak tribe as a spice in cooking. As for China and the Asian plains, Batak onions (A. chinense) or chive or jiaitou (China) are widely used to treat various diseases such as heart disease, headaches, worms, diarrhea, tumors, and antisangga. From the research conducted by Naibaho (2015) it is known that the extract of Batak onions (A. chinense) has antimicrobial activity against Escherichia coli, Staphylococcus aureus, Salmonella typhi, and Bacillus subtilis, and Candida albicans.

Based on the literature study above, until now the antimicrobial research of the Batak onion (A. chinense) plant, especially the antifunginya is still small. Therefore, researchers are interested to see the antifungal effect of Candida albicans ethanol extract on batak onions after it is formulated into liquid soap by varying the concentration of 2%, 3% and 4%.

2 RESEARCH METHODS

The research phase includes preparation of ingredients, characteristics of simplicia, phytochemical screening and extraction. This research was conducted at the Pharmacy Supply Technology Laboratory of Pharmacy Study Program Faculty of Pharmacy, Medical Institute of Lubuk Pakam Medistra used for the process of making liquid soap preparations, and the Microbiology Laboratory of Pharmacy Faculty of Pharmacy Study Program at the Hospital. Grandmed Lubuk Pakam is used for the testing process of antifungal activity. The all processes can be seen in Figure 1.

Furthermore, antifungal activity testing uses the agar diffusion method using disc paper. The parameters observed were the magnitude of the diameter of the inhibition of fungal growth.

This research, tools and materials include: Stirring rods, Beaker glass, Blenders, Spray bottles, Burette, Vaporizer cup, Funnel, Erlenmeyer, Measuring cup, Watch glass, Analytical balance, Water balance, Pycnometer, Dropper, Knife, Rotary evaporator, Spatula, Bunsen, Desiccator, Filter Paper, Refrigerator, Microscope, Tube Clamp, Dropper Pipes, Tube Rack, Rotary Evaporator, Spatula, Test Tubes, Analytical Scales, Autoclaves, Bunsen, Petri Dish, Erlenmeyer, Incubator, Calibration Shaft, Tube Rack, Rotary Evaporator, Spatula, Test Tubes, Analytical Scales, Autoclaves, Bunsen, Petri Dish, Erlenmeyer, Incubator, Calibration Shaft, Rotary Tube , Parchment Paper, Gas Stove, Laminary Air Flow (LAC), Oven, Bedwetting, Tube Clamp, Micro Pipette, Citric Acid, Aquadest, Batak Onion (Allium Chinense), Ethanol 96%, Sodium Chloride, Sodium Lauryl Sulphate, Perfume, Propylenglycol , Distilled water, Batak onions, Potato Dextrose Agar (PDA), Candida albicans Mushroom, Sodium chloride, Liquid soap ethanol extract of Batak onion (Allium chinense). The tools were used in the antifungal activity test are sterilized using an oven at 170 ^{o}C for 1-2 hours and for media sterilized in an autoclave at 121 ^{o}C for 15 minutes.

Sample preparation consists of collecting plants, processing samples, and making extracts. The collection of sample is carried out in a purpose that is without comparing with the same plant from other regions. The sample that is used was Batak onion (Allium chinense) obtained from Siborong-Borong, North Tapanuli.

Processing the sample in advance by cleaning batak onions from dirt by washing under running water until clean then drained to dry after it is sliced into small pieces dried in a drying cabinet / in the room (aerated). Batak onions that have dried are blended until they become powder weighed by weight and are called simplicia. Then simplicia in a blender until it becomes powder, then weighed. Furthermore, simplisa is put into a glass container and stored in a place that is protected from sunlight.

The ethanol extract of batak onions was done by maceration using ethanol 96%. This maceration method was chosen because the method used is simple and the tools used are easy to operate, and do not need intensive supervision. How it works: 500 g sample of onion batak that has been grounded in maceration using 96% ethanol for 3 days while stirring and stored in a place that is not exposed to sunlight. Then the results of the maceration are filtered with a flannel cloth, then the pulp is soaked again for 2 days using 96% ethanol, then filtered again and the pulp is discarded. Then the filtrate results in a rotary evaporator until it becomes a thick extract.

The tools used in the antifungal activity test are sterilized before use. Non-scaled glassware is sterilized in an oven at 170 ^{o}C for 1-2 hours. The media was sterilized in an autoclave at 121 ^{o}C for 15 minutes. Ose needles burned with bunsen lamps (Putri, 2016).

Preparation of ethanol extracts of batak onion test solutions was made with varying concentrations to be tested on test mushrooms, namely 1%, 5% and 10%. Making variation of the extract concentration by dissolving the onion batak in DMSO as much as 10 ml.

Manufacturing media consists of making Media Potato Dextrose Agar (PDA) and NaCl 0.9% solution. PDA, which is 3 g PDA powder weighed, dissolve it in 1 liter of distilled water and heat until it boils until all PDA powder dissolves, then sterilize in an autoclave at 121 ^{o}C for 15 minutes. Sodium chloride weighed as much as 0.9 g then dissolve in sterile aquadest little by little in 100 ml erlenmeyer until completely dissolved, sterilized autoclaved at 121 ^{o}C for 15 minutes.

Mushroom culture consists of making a stock of candida albicans mushroom culture and candida albicans mushroom inoculum. The production of

Candida Albicans mushroom culture stock with a mushroom colony was taken using a sterile ose needle, then implanted on a sloping PDA media. Incubated for 48 hours in an incubator at a temperature of 20-25 ^{o}C. Fungal colonies were taken from solid culture stock with sterile ose needles then suspended in a test tube containing 10 ml of 0.9% NaCl solution. Then measured, turbidity in the solution.

Making concentrations of the ethanol extract of the Batak onions made varying concentrations to be tested on test mushrooms by 2%, 4% and 6%. Making variations of the extract concentration by dissolving the extract of batak onions into a DMSO solvent of 10 ml. Testing the antifungal activity of liquid soap preparations by providing 10 µl of PDA base media was poured into a petri dish and allowed to harden. On the surface of the base layer 6 are placed and arranged so that there is a good area to observe the inhibition zone that occurs. PDA containing 20 µL of candida albicans suspension was poured into a petri dish around the block. The extractor was removed from the petri dish so that a well was formed to be used for all test solution formulas and ethanol extract liquid soap on the batak onion.

Standard formulation for making soap (Apriani, 2016):

R/ Na lauril sulfat	18,5%
NaCl	5ml
Propilen glikol	1g
Asam Sitrat	0,5g
Aquadest ad	100ml

Modification of formulation

R/Na lauril sulfat	18,5%
NaCl	5ml
Propilen glikol	1g
Asam Sitrat	0,5g
Aquadest ad	100ml
Oleum citrus	5ml
Batak onion extract	x

Manufacture liquid soap consists of Na lauryl sulfate added with homogeneous NaCl added citric acid, propylenglycol, partial aquadest and batak onion extract stirred until homogeneous. After all the ingredients are mixed, then add up to 100 ml of distilled water.

Evaluation of liquid soap preparations consists of organoleptic evaluation, homogeneity, and determination of pH values. Organoleptic evaluation is done by observing the appearance of the preparation including the odor, color and texture of the preparation. Homogeneity evaluation was carried out by means of each formula of batak onion liquid soap weighed at 0.1 g. Put on a glass object then observed under a microscope at magnification 100 times. Determination of the pH value of the preparation is done by using a pH meter. The pH check begins with the calibration of the tool using buffer solutions pH 7 and pH 4. A total of 1 g of the soap to be examined is diluted with distilled water up to 10 ml. The pH meter is inserted into the soap solution and wait until the pH meter indicator is stable and shows a constant pH value.

Testing of antifungal liquid doap activity with a total of 10 µl of PDA media was poured into the petri cawn and left to harden. On the surface of the base layer are placed 6 propellers and arranged in such a way that there is a good area to observe the obstacle zone. PDA containing 20 µl candida albicans suspension were poured into a petri dish around the propeller. Remove the propeller from the penthouse cup so that it forms a well that will be used for all test solution formulas and liquid ethanol extract onion extracts.

Form:

$$\text{Water content} = \frac{\text{Water of volume (ml)}}{\text{Weight of sampel (g)}} \text{ x } 100\% \quad (1)$$

Determination of water content in simplicia is done to determine the amount of water contained in the simplicia. The results obtained from the determination of water content, less than 10% is 8.56% can be seen in Table 1. Water content that exceeds 10% can be a good medium for microbial growth, the presence of fungi or insects, and encourage damage to the quality of simplicia in accordance with the Indonesian Materia Medika (MMI).

Table 1: Calculation of determination of water content.

Sample	Weight of Sampel (g)	Volume of Water (ml)	Water content (%)
1	5,10	0,43	8,43
2	5,10	0,43	8,43
3	5,10	0,45	8,82
	Mean		8,56

Determination of water soluble extract content is done to determine the amount of polar compounds that can be found in a water solvent. The water soluble extract content obtained was 18.26% and can be seen in Table 2.

Form :

$$\text{Water soluble essence} = \frac{\text{Weight of essence (g)}}{\text{Weight of sampel (g)}} \times \frac{100}{20} \times 100\% \quad (2)$$

Table 2: Calculation of water soluble essence.

Sample	Weight of Sample (g)	Weight of essence (g)	The essence of water soluble (%)
1	5,010	0,1900	18,96
2	5,025	0,2100	20,89
3	5,020	0,1500	14,94
	Mean		18,26

Determination of ethanol soluble extract levels is done to determine the amount of polar and non-polar compounds that can be found in ethanol solvents. The content of soluble ethanol extract obtained was 14.56% and can be seen in Table 3. Determination of total ash content was carried out to determine the amount of minerals contained in the sample. The total ash content obtained was 5.86%.

Form :

$$\text{Soluble extracts Ethanol} = \frac{\text{Weight of essence (g)}}{\text{Weight of sampel (g)}} \times \frac{100}{20} \times 100\% \quad (3)$$

Table 3: Calculation of water soluble in ethanol.

Sample	Weight of Sampel (g)	Weight of essence (g)	Soluble essence in ethanol (%)
1	5,015	0,1400	16,89
2	5,030	0,1300	12,92
3	5,040	0,1400	13,88
	Mean		14,56

Determination of the pH value of the preparation is done using a pH meter. The pH check begins with the calibration of the tool using buffer solution pH 7 and pH 4. A total of 1 g of the soap to be examined is diluted with distilled water up to 10 ml and can be seen in Table 4. Entering the pH meter into the soap solution is waited until the pH meter indicator is stable and shows a constant pH value.

Form :

$$V1 \times C1 = V2 \times C2 \quad (4)$$

Information:
V1 = the volume of extract solution taken (ml)
C1= concentration of ethanol extract taken (mg/ml)
V2 = the volume of the extract solution made (ml)
C2 = the concentration of ethanol extract made (mg/ml)

Table 4: Calculation of water soluble in ethanol.

Concentration (%)	V1 (ml)	C1 (mg/ml_	V2 (ml)	C2 (mg/ml)
1	10	50	1	100
5	10	50	5	100
10	1 gram ekstract in 10 ml DMSO			

Evaluation of liquid soap preparations consists of organoleptic evaluation, homogeneity, and determination of pH values. Organoleptic evaluation is done by observing the appearance of the preparation including the odor, color and texture of the preparation. Homogeneity evaluation was carried out by means of each formula of batak onion liquid soap weighed at 0.1 g. Put on a glass object then observed under a microscope at magnification 100 times. Determination of the pH value of the preparation is done by using a pH meter. The pH check begins with the calibration of the tool using buffer solutions pH 7 and pH 4. A total of 1 g of the soap to be examined is diluted with distilled water up to 10 ml. The pH meter is inserted into the soap solution and wait until the pH meter indicator is stable and shows a constant pH value.

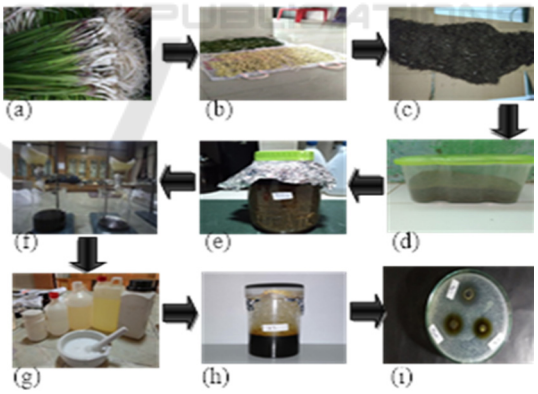

Figure 1: Data Collection Process: (a) Fresh batak onions as much as 10 kg, (b) Cut into small pieces, then dried by aerating for 14 days, (c) Dried simplisia, (d) Simplicia batak onions that have been mashed, (e) Maceration prosess by using 96 % ethanol for 5 days, (f) The simplicia extract screening process, (g) Soap making process, (h) Liquid soap preparations with simplisia extract, (i) Liquid soap results test activity.

3 RESULTS

The results obtained from the ethanol extraction process of Batak onions (Allium chinense) using 96% ethanol solvent concentrated with a rotary evaporator at a temperature of ± 78ºC which is in the form of a thick extract of 500 gr. The resulting extract has a aroma of onion and brown can be seen in Table 5.

Table 5: The result of batak onion extraction.

Extraction	Descriptions		
	Color	Smell	Shape
I	Brown	Batak onion smell	Thick
II	Brown	Batak onion smell	Thick

Organoleptic Test Results show that the liquid soap base without the addition of white onion extracts while the addition of the extract produced dark brown liquid soap preparations because the extract was added to the dark brown liquid soap base. The intensity of the color of the liquid soap preparation increases with the increasing concentration of the extract added. All liquid soap preparation formulas that are made produce a soft and homogeneous consistency. The results can be seen in Table 6.

Table 6: Organoleptic results.

Formulation	Texsture	Smell	Color	Homogen
Liquid soap base	Soft	No	White	Homogen
F1	Soft	Batak onion smell	Brown	Homogen
F2	Soft	Batak onion smell	Brown	Homogen
F3	Soft	Batak onion smell	Dark Brown	Homogen

Information: F: Formulation, F1: 1%, F2: 5% and F3; 10%.

Determination of ethanol soluble extract levels is done to determine the amount of polar and non-polar compounds that can be found in ethanol solvents. The content of soluble ethanol extract obtained was 14.56%. Determination of total ash content was carried out to determine the amount of minerals contained in the sample. The total ash content obtained was 5.86%. The resulting of the examination of the characteristics soap can be seen in Table 7.

The results of the antifungal activity test for liquid soap ethanol extract on Batak onion show that the basic formula of liquid soap tested soap could not inhibit the growth of Candida albicans mushrooms, ethanol extract of Batak onions against inhibition of Candida albicans fungus on DMSO did not show any inhibitory, ethanol extract of Batak onion liquid soap which had the highest activity in inhibiting the growth of Candida albicans mushrooms was 10% with inhibition zones of 23.67 mm. Whereas on ethanol extract liquid soap on batak 5% the inhibition zone is 22.27 mm and ethanol extract liquid soap on the batak concentration is 1% the lowest inhibitory zone. The resulting of the Inhibitory zone of ethanol extract liquid soap can be seen in Table 8.

Table 7: The results of the examination of the characteristics of the simplicia onion batak powder.

No.	Parameter	Result (%)
1.	Water content	8,56
2.	Water Soluble essence Content	18,26
3.	Ethanol Soluble essence Content	14,56
4.	Total Ash Content	5,86

Table 8: Inhibitory zone of ethanol extract liquid soap against the growth of candida albicans.

No.	Liquid Soap Concentration of Batak Onion Extract (%)	Inhibitory Zone Diameter (mm)			Mean
		I	II	III	
1.	DMSO	-	-	-	-
2.	1%	21,0	21,5	21,3	21,26
3.	5%	22,5	22,2	22,1	22,27
4.	10%	23,9	23,7	23,4	23,67

Information :

- = not inhibit,
% = concetration % (mg/ml)

According to the literature the inhibition diameter with a weak activity is 10-15 mm, the diameter of the active activity is 16-20 mm, and the diameter of the inhibitor with strong activity is> 20 mm (Greenwood, 2018).

The results of the chemical compound group showed that the ethanol extract of the batak onions contained a class of chemical compounds in the form of flavonoids, saponins and steroids / triterpenoids. These secondary metabolites have antifungal activity with different mechanisms of action. Phenol or polyphenol compounds are the largest group of secondary metabolites that have antifungal antivens,

have hydroxyl groups attached to aromatic compounds. The location and number of hydroxyl groups in phenol compounds affect the toxicity of microorganisms. The combination of phenol compounds can provide a synergistic effect and increase antifungal reactions better than a single compound. Phenol compounds at low concentrations affect enzyme activity, whereas at high concentrations cause protein denaturation (Suprihatin, 2017).

Flavonoids are chemical compounds that have potential as antifungals. Flavonoids are a group of phenol compounds that have a tendency to bind to proteins, thus interfering with the process of fungal metabolism, besides flavonoids also function as antifungals by forming complex compounds against extracellular proteins that interfere with fungal cell membranes (Setyowati, 2017; Widyasanti, et all, 2017).

The mechanism of terpenoids as an antifungal is to react with porin (transmembrane protein) on the outer membrane of the fungal cell wall, forming a strong polymeric bond, causing damage to the porin. Terpenoid compounds are easily soluble in lipids, this is the nature that causes these compounds to easily penetrate the fungal cell wall (Enzo Palese, Maurizio Nudo, Grazia Zino, et al., 2018). The mechanism of steroids as antifungals is related to lipid membranes and the sensitivity of steroid compounds that can cause leakage of lymphosomes (Jahani, S. Bazi, S. Shahi, Z. Sheykhzade, A, 2017).
Saponins are included in the antifungal group which interferes with the permeability of fungal cell membranes. The mechanism of action of saponins as an antifungal is to reduce surface tension resulting in increased cell permeability or leakage and cause intracellular compounds to exit the cell (Ria, 2017).

4 CONCLUSION

The results of macroscopic examination revealed that the onion batak has a slender leaf shape, in terms of 3 to 5, has a length of 45-50 cm. tubers have white, grayish white and purplish white with a length of 4-5 cm, smell and crisp texture. While the results of microscopic examination are known that the examination of the simplicia powder on the batak showed the presence of parenchyma, sclerenkim and essential oils.

Ethanol extract of Batak onion (Allium chinense) can be formulated as liquid soap and has antifungal activity.

Ethanol extract liquid soap preparations in concentrations of 1% with inhibition zone diameter of 21.26 mm, at concentrations of 5% inhibition zone 22.27 mm and antifungal activity of ethanol extract of batak onions that have the most effective antifungal activity against candida albicans fungus at a concentration of 10% with inhibition zone diameter 23.67 mm has antifungal activity in inhibiting the growth of Candida albicans. Then the results obtained from ethanol extract of batak onion soap can be used as an antifungal Candida albicans.

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