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Isolation of Triterpenoids Onion Sabrang (*Eleutherine bulbosa*) and Antibacterial Activity Tests of *Escherichia coli*

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Abstract

Research on the isolation and antibacterial activity test of the Sabrang Onion (*Eleutherine bulbosa*) bulb has been carried out. This study aims to obtain triterpenoid compounds from Sabrang onion bulb and find out its activity as an antibacterial against Escherichia coli bacteria causing Urinary Tract Infection (UTI). UTI is a disease with a fairly high prevalence of 80% caused by *E. coli* bacteria, with the highest number of patient are woman. The stages of this research include extraction by maceration method using n-hexane solvent. The concentrated n-hexane extract was then analyzed with thin layer chromatography. Positive identification of triterpenoids was carried out using Liebermann-Burchard reagents with n-hexane: ethyl acetate (9: 1) eluent. The obtained isolates were also analyzed using UV-Vis spectrophotometry. The results of the analysis showed that the isolate had an absorbance value of 0.205 A at a wavelength of 443 nm and 0.430 A at a wavelength of 788 nm. The isolates were tested for antibacterial activity against *E. coli* bacteria by agar diffusion method. The results showed that the inhibition zone diameter at a concentration of 0.5% w / v was 20.19 mm, at a concentration of 1% w / v the inhibitory zone was 21.83 mm. In the positive control used standard tetracycline, the diameter of the inhibition zone was 40.67mm. Inhibition zone diameter at a concentration of 0.5% w / v was 20.19 mm, at a concentration of 1% w / v the resulting inhibition zone was 21.83 mm. An increase in the response of inhibition against *E. coli* bacteria with a concentration of 1% w/ v of 1.64 mm. In the positive control, the diameter of inhibition zone is 40.67 mm. Thus, triterpenoid isolates from Sabrang Onion are effective as antibacterial, in the strong category with inhibition zone diameters > 20 mm.

INTRODUCTION

Sabrang onion (*Eleutherine bulbosa*) from the family Iridaceae is one of the plants that are widely used by the community as medicine, where the parts used are bulb (Daryono et al., 2013). Secondary metabolites contained in sabrang onions include flavonoids, phenols, glycosides, anthraquinones, and triterpenoids. Based on research by Shai et al., (2008), triterpenoids are known to have significant antibacterial properties. In the inhibition zone testing of *Staphylococcus aureus* (Gram-positive) bacteria, 100 ≤MIC ≤ 512 µg / mL results

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Warnida et al., (2016) were obtained. Triterpenes are widely distributed in marine plants and animals, either free, as esters, or as glycosides (saponins) (Awolola et al., 2014; Catteau et al., 2018). Triterpenes consist of six isoprene (C_5H_8) 6 (six) units of mevalonic acid or deoxicellulose phosphate, and 1 comes from the reduction of two farnesyl pyrophosphate molecules by squalen synthase. Triterpenoids are usually classified into three groups namely acyclic, tetracyclic and pentacyclic Catteau et al., (2018).

The results of phytochemical screening for simplicia and ethanol extracts of Sabrang onions showed that the onions contain alkaloids, glycosides, flavonoids, phenolics, steroids, triterpenoids which are potential biopharmaca sources to be developed as modern medicinal plants in human life because they have potential as antioxidants and antibacterials (Sa'adah et al., 2016). Other phytochemical test results showed that sabrang onion tuber extract can inhibit the growth of many bacteria, including *Bacillus subtilis*, *Streptococcus pneumoniae*, *Staphylococcus epidermis*, *Escherichia coli*, *Micrococcus luteus*, *Methicillin-Resistant Staphylococcus Aureus* (MRSA), *Propionibacterium acnes*, *Salmonella typhi*, and *Staphylococcus aureus* (Sharma et al., 2014).

According to Korbel et al., (2017); Okarska-Napierała et al., (2017), urinary tract infections (UTI) are one of the most commonly diagnosed infections in children and the elderly. The incidence of UTI is 1: 100 per year. The incidence of UTI increases in children, decreases in adulthood, and increases again in the elderly. More than 10% of women > 65 years old reported experiencing a UTI in the last 12 years. This number increased by almost 30% in women age > 80 years. According to the Ministry of Health (2015) the number of UTI patient in Indonesia is 95 cases / 104 residents per year or around 180,000 new cases per year (Masajtis-Zagajewska & Nowicki, 2017).

Infection can occur through inflammation of the prostate gland, inflammation of the bladder urine, and inflammation that results injury in the urethra (Bjorling et al., 2011; Heyns, 2012). The presence of UTI is characterized by suprapubic pain, dysiuria, hematuria, urgency, and starguria, some are even accompanied by fever, vomiting and back pain. Some research results show that *Escherichia coli* bacteria cause 80-95% of UTI (Nielsen et al., 2014; Widianingsih & De Jesus, 2018).

According to Piranfar et al., (2013), UTI is the most commonly acquired infection in the world community and the most common pathogen is *E. coli*. *E. coli* causes UTI without complications by 80%, therefore antibiotic selection needs to be considered to prevent resistance. In 2011, infectious disease patient in the United States recommended the use of trimethoprim-sulfamethoxazole in treating UTIs. However, the level of resistance of *E. coli* to most antibiotics is increasing worldwide (Alonso et al., 2017). Therefore, it is necessary to look for other alternatives for the treatment of UTIs in the form of antibacterial from natural ingredients such as safe onions and minimal side effects while increasing the effectiveness of the use of sabrang onions as natural medicines (Sabra et al., 2010; Saifuddeen et al., 2014; Sharma et al., 2014).

METHODS

This research was conducted through experiments in the microbiology laboratory and laboratory research center at the Faculty of Pharmacy, Universitas Andalas, Indonesia. The sample used was a sample of dried Sabrang onion bulbs taken randomly (Sabra et al., 2011; Sabra et al., 2010). Samples were washed, chopped and then air dried for 7 days in a greenhouse. After drying, the sample is grinded using a grinder to obtain dry powder. Extraction A total of ± 500 g of dried powder of Sabrang onion bulbs was extracted by maceration technique for 72 hours using n-hexane solvent. The n-hexane extract obtained was separated from the solvent and then concentrated using a rotary vaccum evaporator. The viscous extract was tested for phytochemistry by Liebermann-Burchard reagent to determine the presence or absence of triterpenoids. Positive isolates contain yellow triterpenoids.

Qualitative Test of Triterpenoid

Triterpenoid positive viscous extracts were separated by thin layer chromatography using silica gel 60 as stationary phase, and n-hexane: ethyl acetate 9:1 as mobile phase (Angelis et al., 2017). The result of the separation of thin layer chromatography with Liebermann-Burchard stain appearance was tested positive for triterpenoids if there was a single purplish blue stain. The isolates obtained from the TLC results suspected to be triterpenoid compounds were analyzed by UV-Vis spectrophotometer (Fabri et al., 2014). A total of 2 ml of triterpenoid isolates from TLC separation dissolved in n-hexane were put in cuvettes up to a half and analyzed with a wavelength range of 200-800 nm. In the blank, the n-hexane solvent was put into a half cuvette and analyzed using a spectrophotometer in the wavelength range of 200-800 nm. The formed spectrum is observed and recorded the wavelength and absorbance at the peak formed (Behera & Acharya, 2008; S. Hu et al., 2015).

Antibacterial Test

Bacterial rejuvenation test

The *E. coli* bacteria obtained from the Sumatran Biota Laboratory was inoculated into the sloping medium by taking as many as one eyepiece aseptically then inoculated by scraping on the sloping agar medium. Subsequently incubated for 24 hours at 37 ° C until cropping occurs.

Preparation of bacterial culture suspensions

E. coli culture in Nutrient Agar (NA) media was taken aseptically as much as one ose, then put in 50 mL of NA media and shaker until homogeneous.

Preparation of test samples

The n-hexane isolated compound was made of 0.5% w / v and 1% w / v using DMSO solvent.

Antibacterial activity test

The antibacterial activity test was carried out using the diffusion method to use 5 mm diameter paper discs with *E. coli* test bacteria. Disc paper was dropped with triterpenoid isolates whose concentrations were 0.5% w / v and 1% w / v which were then placed on inoculated NA media. Standard tetracycline as a positive control (20mg / ml). Incubation was carried out at 37 ° C for 1 x 24 hours. Observations were made on the formation of inhibitory zones around the paper disc.

RESULT AND DISCUSSION

On screening the sabrang onions obtained reddish brownish red powder as much as 2.05 grams with a soft texture. Tests using thin layer chromatography found a spot of purplish-blue which indicates a positive sample containing triterpenoids. The test results using UV-Vis spectrophotometry showed a sharp peak intensity on the spectrum. Furthermore, in antibacterial testing using the diffusion method in order to obtain the inhibition zone diameter of 20, 19 mm in samples with a concentration of 0.5% w / v, 21, 83 mm at a concentration of 1% w / v, and 40, 67 mm in the positive control namely standard tetracycline.

Fig.1. Triterpenoid Isolate Sabrang Onion

Fig.2. Spot of Purplish-blue

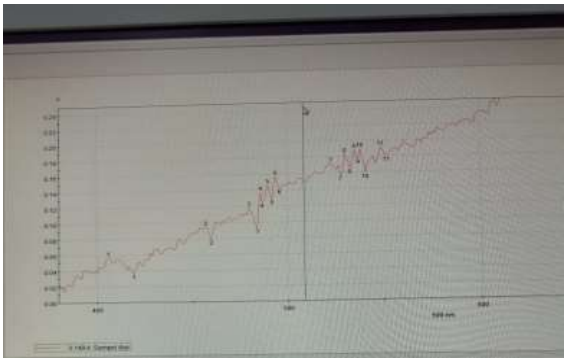

Fig.3. Spectrum with sharp peaks

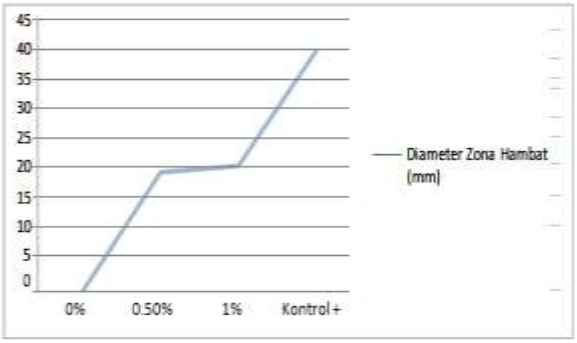

Fig.4. Graph of Relationship between

On screening the sabrang onions obtained reddish brownish red powder. Extraction is done using the method of immersion (maceration) (Mukhtar et al., 2018). Maceration method was chosen because it has advantages such as cheap, simple procedure, and can avoid damage to compounds that are not resistant to heat, because this method is done without a heating process. Maceration was carried out with n-hexane as a solvent to obtain secondary metabolites of triterpenoids in the bulbs of Sabrang onion. n-hexane is a non-polar solvent, so that it can easily dissolve triterpenoid compounds which are non-polar compounds, based on the principle of like dissolves like, where polar solvents can only dissolve polar compounds and non-polar compounds can only dissolve non-polar compounds. n-hexane extract from maceration results in a yellow liquid. Furthermore, the extract was concentrated using a rotary evaporator so that a viscous extract of 2.05 grams was obtained with a soft texture. The sample yield obtained by 0.41% (K. Hu et al., 2018; Trigui et al., 2018).

The n-hexane viscous extract obtained was then tested qualitatively by thin layer chromatography with n-hexane: ethyl acetate (9: 1) eluent (Paillat et al., 2012; Liu et al., 2018). Viscous extract was used as much as 10 ppm dissolved in n-hexane. Then the extract is given a Liebermann-Burchard stain viewer, because the triterpenoid does not have a chromophore group, so it cannot be observed directly under UV light. The plates were observed under UV light at wavelengths of 286 nm and 366 nm. The results show a purplish blue stain which is a triterpenoid.

Triterpenoid isolates were then tested for purity with a UV-Vis spectrophotometer (Awolola et al., 2014). In determining the maximum wavelength, values of 443 nm - 788 nm are obtained. The analysis showed an absorbance value of 0.205 A in length waves 443 nm and 0.430 A at a wavelength of 788 nm, and there is a peak with a sharp intensity. Testing the activity of triterpenoid isolates against *E. coli* bacteria was carried out by the agar diffusion method, to determine the inhibitory zones produced by the isolates. The results showed that the inhibition zone diameter at a sample concentration of 0.5% w / v was 20.19 mm, at a concentration of 1% w / v the resulting inhibition zone was 21.83 mm. In the positive control used standard tetracycline, the diameter of the inhibition zone was 40.67 mm. The response to bacterial growth inhibition can be classified as follows:

Tabel 1. Response To Bacterial Growth Inhibition

Inhibitory Zone Diameter	Growth Inhibition Response
>20 mm	Strong
16-20 mm	Medium
10-15 mm	Weak

Based on the relationship graph sample concentration with the diameter of the bacterial inhibition zone shows the diameter of the inhibitory zone at a concentration of 0.5% w / v of 20.19 mm, at a concentration of 1% w / v of the inhibitory zone produced by 21.83 mm. An increase in the response of inhibition against *E. coli* bacteria with a concentration of 1% w / v of 1.64 mm. The positive control used is standard tetracycline, inhibition zone diameter of 40.67 mm. Thus, triterpenoid isolates from Sabrang Onion are

effective as antibacterial, included in the strong category with inhibition zone diameters > 20 mm.

CONCLUSION

In conclusion, the isolation of triterpenoid compounds from Sabrang Onion *(Eleutherine bulbosa)* at concentrations of 0.5% w/v and 1% w/v has demonstrated significant effectiveness in inhibiting the growth of *Escherichia coli bacteria*, a major contributor to urinary tract infections. The observed inhibition zone exceeding 20 mm indicates a robust antibacterial activity, categorizing the isolated triterpenoids as potent agents against *E. coli*. These findings underscore the potential of Sabrang Onion as a valuable source for the development of antimicrobial agents, particularly in addressing bacterial infections associated with urinary tract health. Further exploration and research into the molecular mechanisms underlying this inhibitory effect could contribute to the development of novel therapeutic strategies.

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