Proceeding of The 1st University of Muhammadiyah Purwokerto - Pharmacy International Conference
5-6 June 2015, Horison Hotel Purwokerto
ISBN 978-602-73538-0-0

In Vitro Antibacterial Test of Soursop (*Annona muricata* Linn) n-Hexane Extract Leaves on *Propionibacterium Acnes*

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ABSTRACT

One of the plants that have potential as an antibacterial is soursop (*Annona muricata* Linn). This study was aimed to determine the antibacterial activity of soursop leaves against *Propionibacterium acnes* using n-hexane solvent. The results showed that of n-hexane extract of soursop at concentrations of 1000 ppm, 2000 ppm, 3000 ppm and 4000 ppm inhibit the growth of *P. acnes* bacteria with diameter of inhibition zone of 9.167 mm; 12.088 mm; 12.340 mm; 21.217 mm, respectively. The formed inhibition zone was greater with higher concentrations of n-hexane extract of the leaves of the soursop.

Keywords: antibacterial, inhibitory zone, n-hexane extract, *P. acnes*, soursop leaves.

INTRODUCTION

Infectious disease is one of the ever growing problems in health field. Infection is a condition that microorganisms enters the body, multiply and cause disease. This situation can be viewed as a type of parasitism happens as an organism living at the expense of another organism, namely the host. The parasites multiply and metabolically active in the host's body (Hoan and Rahardjo, 2002). Infectious diseases can be caused by four large groups namely bacteria, fungi, viruses, and parasites (Jawetz et al., 2001).

Propionibacterium acnes is included in the group Corynebacteria. It is a normal bacterial flora of the skin. *P. acnes* play a role in the pathogenesis of acne by producing lipases that break free fatty acids from the skin lipid (Brook et al., 2005). These fatty acids can lead to tissue inflammation when connected to the immune system further support the occurrence of acne. Acne occurs when the skin pores clogged. The pores are holes for a channel called as follicle, which contains hair and oil glands. Normally, the oil glands help keep the skin moist and remove dead skin cells. When the oil glands produce too much oil, the pores will be overstocked with dirt and bacteria. The mechanism of acne is *P. acnes* bacteria damage the stratum corneum and stratum germinate by means of secreting chemicals that destroy the pore walls. This condition causes inflammation. Skin fatty acids and oils are clogged and hardened. If acne is touched, the inflammation will be expanded and the solid fatty acids and oils are hardened so that skin will dilate (Anonymous, 2007).

Drugs that are used for topical treatment contains sulfur and astringent. Benzoyl peroxide 2.5-10% is very active in the fight against *P. acnes*. These drugs are comedolytic, as it contains

ISBN 978-602-73538-0-0

antimicrobial, anti-blackhead, and anti-inflammatory effects, however, its main disadvantage is that it can cause irritation. Topical erythromycin and clindamycin are also just as effective as benzoyl peroxide. Systemic therapy drugs used are tetracycline and erythromycin. However, to use the gastrointestinal system in use on an empty stomach will cause bad effects.

Many attempts have been made to overcome the problems posed by the treatment of synthetic drug, namely by finding a compound that can kill and inhibit the bacteria naturally. One of the plants that have the potential as a natural antibacterial agent is soursop (*Annona muricata* L.). A study conducted by Phatak (2010) showed that the methanol extract of soursop leaves contain steroids, terpenoids, and tannins compound that can inhibit the growth of *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhimurium* and *Klebsiella Pneumonia*. Therefore, this study tested the antibacterial activity of n-hexane extract of soursop leaves against *P. acnes*.

EXPERIMENTAL

2.1 Extraction

Soursop leaves are washed thoroughly so that impurities such as dust on the leaves of the soursop are lost. Further soursop leaves samples were dried out of sunlight. Dry Soursop leaf was powderized to broaden contacts and improve its interaction with the solvent. Extraction was performed by maceration using n-hexane solvent. A total of approximately 100 grams of soursop leaves were soaked with 650 ml of n-hexane, closed and then stored in a dark room and shaken at 120 rpm for one week. Afterward, the filtrate was taken and the residue was re-macerated with 50 ml of n-hexane. The filtrate was taken every day for three days. After three days, or after the color produced clear, maceration process was terminated. The filtrate obtained was concentrated using rotary evaporator at a temperature of ± 40 °C. The concentrated extract is weighed.

2.2 Regeneration Bacteria Test

Bacteria used to test the antibacterial should be regenerated before use. Bacteria stock which is the primary culture was initially cultured in an agar slant (Nutrient agar), as many as one ose bacteria was carved into the x NA slant media and was incubated at 37 ^{o}C for 24 hours. The culture is the initial activity of the stock of bacteria which was then stored at a temperature of 5 ^{o}C.

2.3. Antibacterial Activity Test

Initial antibacterial activity test was carried out by diffusion. As many as one ose bacteria was taken from the stock culture and was incubated in 10 ml of liquid medium (Nutrient Broth) for 18-24 hours at 37 °C while shaken using a water bath at 100 rpm. Afterward about 5 ml of

ISBN 978-602-73538-0-0

bacterial culture was taken and its OD was measured with a value of less than one at a wavelength of 620 nm. If the OD values are > 1 the culture was taken as much as 50 mL, when OD <1 a 100 mL culture would be taken, and then was distributed in a sterile petri dish. Further, a 15 mL of Nutrient Agar (NA) media at temperature of ± 40 °C was poured and then the cup was shaken so that the bacteria spread evenly. After the result solidified it was riddled with ± 8 mm diameter using a crock drill. As much as 50 mL leaf extract was inserted into the hole with a concentration of each fraction was used for the inhibition test at 1000 ppm and each sample was dissolved in distilled water and then incubated at 37 °C for 24 hours. A clear zone which was visible in around the hole indicated the existence of antibacterial activity in the sample. The formed clear zone was measured using a caliper (Ningsih et al., 2014).

RESULTS AND DISCUSSION

The soursop leaves were taken from the Langkap village Purbalingga. They were first cleaned before maceration. Soursop leaves are dried without being exposed to the sun. Afterward the soursop leaves were grind into powder using blender. Soursop leaves and soursop leaf powder can be seen in Figure 1.

Fig 1. Sample and soursop leaf powder.

The extraction of dried leaf powder soursop was performed by maceration with n-hexane for 7 days and was followed by a concentration phase using rotary evaporator. In the maceration process, soursop leaf cell undergo gripped condition, so that the cell will release active compounds which are then bound by the solvent. Maceration is a technique for sample extraction that is carried for sample that is not heat resistant to the heat by soaking in certain solvents for a certain time. This technique have several advantages such as the simplicity of equipment used is, it only takes a soaking vessel to produce a good product, with this technique

ISBN 978-602-73538-0-0

non heat resistant substances will not be damaged (Harborn, 1987). The results of maceration and concentration of soursop leaf powder extract obtained as much as 100 grams of n-hexane which is solid green and pasta shaped in the amount of 3 grams. Based on these data the percentage gains generated of soursop leaf extract is 3%. The obtained n-hexane extract was used in the antibacterial activity test using agar diffusion method.

P.acne bacterial isolates was obtained from the Laboratory of Microbiology UNSOED. Before being used for antibacterial activity test, bacteria P. acne was rejuvenated advance on media NA slant and was incubated at 37 ^{0}C for 24 hours. In the antibacterial activity test using agar diffusion method *P. acnes* bacteria inoculum was grown in medium Nutrient Broth (NB) 10 ml that was incubated for 24 hours. NB inoculum absorbance was then measured at a wavelength of 620 nm with the absorbance values measured at 1.473 so that inoculum was propagated in Nutrient Agar (NA) media of 50 µl.

The antibacterial activity test was performed using diffusion method in order to determine whether there is influence of soursop leaf extract in inhibiting the growth of *P. acnes* bacteria. It can be seen from the large diameter inhibition zone formed around the hole. The test results of initial antibacterial activity beginning to produce data as shown in Figure 2.

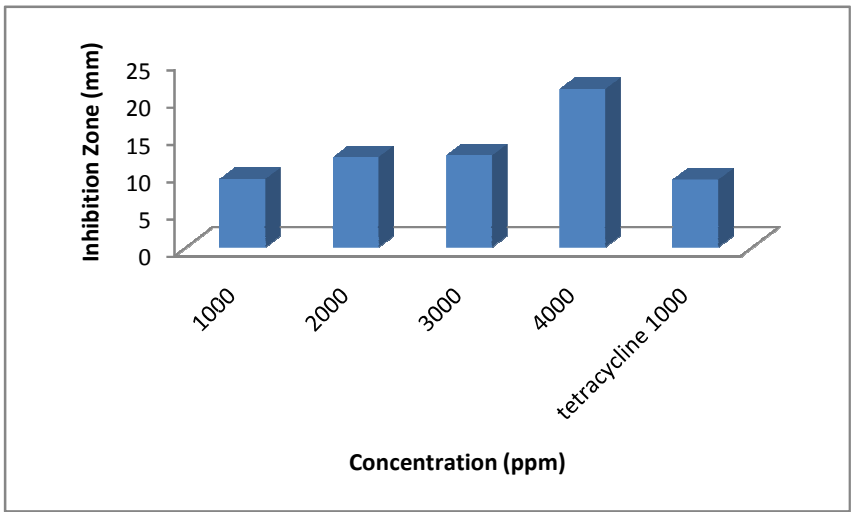

Fig 2. It shows activities of n-hexane extract of soursop leaf against *P. acnes.*

Based on the research results as shown in Figure 2, the greater the concentration of the extract, the greater inhibition zone diameter that was formed, so it can be concluded that both have a proportional relationship to each other. Antibacterial activity of n-hexane extract decreases with decreasing concentration. The positive control used in the determination of minimum growth inhibition concentration is tetracycline with a concentration of 1000 ppm. The result showed that tetracycline at a concentration of 1000 ppm give inhibition zone against *P. acnes* of 12 mm. The

ISBN 978-602-73538-0-0

results obtained shows that n-hexane extract of soursop leaves with concentration of 2000 ppm, 3000 ppm, 4000 ppm had a greater inhibitory zone than tetracycline.

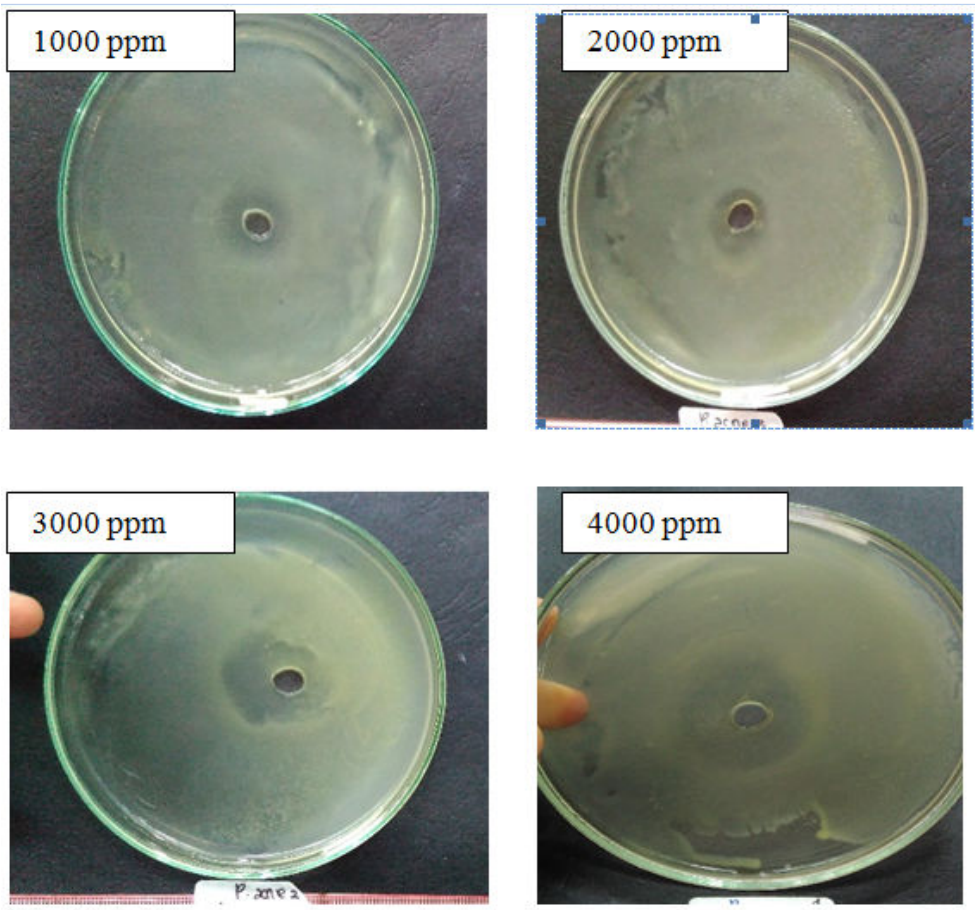

Fig 2. Antibacterial activities of n-hexane extract of soursop leaves against *P. acnes.*

CONCLUSIONS

Based on the results of this study, it can be concluded that the administration of n-hexane extract of leaves of the soursop gives a significant effect in inhibiting the growth of bacteria *P.acne* in vitro. Increasing concentration of n-hexane extract of the soursop leaf give greater inhibition zone.

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