Antibacterial activity of ethanol extract, n-hexan, ethyl acetate and butanol fraction of *Momordica charantia* L. seed against *Staphylococcus epidermidis*

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Abstract. Acne is a skin disease caused by excessive production of oil, causing clogged pilosebaceous follicular ducts and inflammation. One of the bacteria triggered acne is *Staphylococcus epidermidis*. Acne treatment usually uses antibiotics that inhibit inflammation and bacterial growth. To reduce the effects of using chemical drugs, research is needed to find natural treatment solutions such as using the bitter melon plant. The part that is often used from bitter melon is the flesh of the fruit, while the seeds are only used as waste, so researchers are interested in examining the antibacterial activity of bitter melon seeds. Tests were carried out on ethanol extract, fraction of n-hexane, ethyl acetate and butanol in bitter melon (*Momordica charantia* L.) seeds against *Staphylococcus epidermidis* by determining the minimum bactericidal concentration (MBC) using the liquid dilution method of a concentration of 80%; 40%; 20%; 10%; 5%; 2.5%; 1.25% and 0.625%. The value of minimum bactericidal concentration (MBC) is indicated by the absence of colony growth on agar media. The minimum bactericidal concentration (MBC) in this study was only obtained in the ethyl acetate fraction, at a concentration of 40%, while in extracts and other fractions the MBC value was not obtained.

1. Introduction
Indonesia is a country with a tropical climate that causes fertile soil, so that many types of plants can grow and among these types of plants there are several plants that have medicinal properties. Medicinal plants have been used by Indonesian society from hundreds of years ago, either based on experience or knowledge [1]. One of the plants that is widely known by the public and has medicinal properties is bitter melon (*Momordica charantia* L.). Pare is a family of Cucurbitaceae whose often used as a vegetable. It taste is bitter. Bitter melon has many benefits for health and medicine. Bitter melon is rich in protein, fatty acid, carbohydrates, calcium, phosphorus, iron, vitamin A, vitamin B1 and vitamin C. So that bitter melon has many benefits for the body such as diabetes drugs, overcoming asthma, losing weight, controlling cholesterol, anti-cancer, suppresses the development of the HIV virus, improves digestion, and maintains skin beauty [2].

The part that is often used from bitter melon is the flesh of the fruit, while the seeds are only used as waste, so researchers are interested in examining the benefits of bitter melon seeds. In previous research, it was known that bitter melon seed extract had secondary metabolic content. Those are alkaloids, saponins and terpenoids/steroids [3]. Secondary metabolite compounds such as saponins and steroids (triterpenes) are triterpenoids which have antibacterial activity [4]. Antibacterial is a substance that can interfere with the growth or kill bacteria by disrupting bacterial metabolism [5]. So do the bacteria that trigger acne growth such as *Propionibacterium* acnes and *Staphylococcus* epidermidis.
Acne is a skin disease caused by the excessive production of sebaceous glands. It causes the pilosebaceous follicular ducts blocked causing inflammation which is characterized by the eruption of papules, pustules, nodes, and cysts in places such as the face, neck, upper arms, and chest [6]. In normal skin, dust and dead skin cells often accumulate due to lack of care and maintenance, especially to the skin that has a high level of oil production. As a result, the hair follicles become clogged and produce blackheads. Dead skin cells and dirt are exposed to acne-causing bacteria, causing acne. Untreated acne will develop red swelling (papules). If the inflammation gets worse, the white blood cells will rise to the surface of the skin in the form of pus (pus), the acne is called pustus [7]. Acne treatment usually uses antibiotics that can inhibit inflammation and bacterial growth. For example, tetracyclines, erythromycin and clindamycin.[8] To reduce the effects of using chemical drugs, it is necessary to conduct research to find natural healing solutions using plant parts, one of the plants that can be used is bitter melon.

This antibacterial activity test uses the dilution method. The dilution method used to determine the ability of a compound against bacterial or fungal activity, this method is useful for finding out how much anti-microbial substances are needed to inhibit growth or kill tested bacteria or fungi [9]. Another study showed that the antibacterial activity of bitter melon extract and fraction against Propionibacterium acnes obtained MIC value from the extract at a concentration of 30%. Based on the description above, research was carried out on the antibacterial activity of ethanol extract, n-hexane fraction, ethyl acetate and butanol of bitter melon seeds against Staphylococcus epidermidis with a concentration of 80%; 40%; 20%; 10%; 5%; 2.5%; 1.25% and 0.625% using the liquid dilution method.

2. Methods

The tools used in the research were meseration bottles, funnels, rotary evaporators, analytical scales, petri dishes, racks and test tubes, measuring cups, beaker glass, watch glasses, stirring rods, ovens, Fornes Tweezers, sterile cotton, sterile sticks, cloths, gauze, parchment paper, loop needles, spiritus lamp, erlemenyier, separating funnel, micro pipette, incubator, autoclave, Bunsen, photographic tool, micro pipette, suction pump, spatel, vortex, L rod, matches.

The materials used in this study were bitter melon seeds obtained, 70% ethanol, n-hexane, ethyl acetate, butanol, chloroform, ammonia chloroform, sulfuric acid, acetic acid, HCl, mayer reagent, reagent FeCl3, norite, Mg powder, sodium broth, mueller hinton, DMSO, sterile distilled water, physiological NaCl and Staphylococcus epidermidis bacteria (obtained from the Laboratory of Microbiology, Faculty of Medicine, University of Andalas).

Sample identification was carried out at the Herbarium of the Biology Department, Andalas University (UNAND) Padang

2.1. Preparation for extraction and fractionation

The extract is made by maceration where the sample is put into a dark bottle protected from sunlight and soaked with 70% ethanol until completely immersed for 3-5 days while stirring occasionally, then filtered so that the filtrate is obtained, the dregs are shifted back, do it until the macerate is gone. color again, all the masure is put together and concentrated with a rotary evaporator until a thick extract is obtained. Some of the viscous extract fractionated with a solvent of different polarity, the fractionated extract with hexane was put into a separating funnel, add distilled water and shake, until the hexane layer and water layer were obtained, the hexane layer was then evaporated until thick, while the water layer was continued for the next fraction added with The ethyl acetate was put into a separating funnel and shaken, until a layer of ethyl acetate and a layer of water was obtained, the ethyl acetate layer was evaporated to obtain a viscous fraction of ethyl acetate. The water layer was then continued for the butanol fraction as in the previous step until the viscous butanol fraction was obtained.

Examination of Ethanol Extract and Pare Seed Fraction (Momordica charantia L.) Observations are made visually by observing shape, taste, color and flavour.
2.2. **Phytochemical test**
The extract and fraction of bitter melon seeds weighed 0.5 grams added by 5ml of aquadest and 5ml of chloroform, then shake vigorously and let it form two layers, namely a layer of water and chloroform. The water layer is used for testing flavonoids, phenolics, saponins, and chloroform coating for testing terpenoids, steroids, and alkaloids.

2.2.1. **Flavonoid test** ("Cyanidine Test" Method)
Take 1-2 drops of water layer, drop it on a drop plate then add Mg and HCl (P) powder. The formation of a red color indicates the presence of flavonoids.

2.2.2. **Phenolic test**
Take 1-2 drops of water layer, drop it on the drop plate then add FeCl₃ reagent. The formation of a blue color indicates the presence of phenolic content.

2.2.3. **Saponin test**
Take a layer of water, then shake it vigorously in a test tube, the formation of permanent foam (± 15 minutes) indicates the presence of saponin content.

2.2.4. **Alkaloid test** ("Culvenore-Fristgerald" Method)
Take a little layer of chloroform, then add 10ml chloroform ammonia 0.05 N, stir gently add a few drops of H₂SO₄ 2N then shake gently, let it separate. Taken again, the water layer is put into the test tube (acid layer) add a few drops of mayer reagent, the positive reaction of the alkaloid is indicated by the presence of a white fog to a white lump.

2.2.5. **Terpenoid test**
Take a little layer of chloroform filtered with norit, then put it in a drop plate and let it dry, add 2 drops of H₂SO₄ (P), and add acetic acid, the formation of a blue-purple color indicates the presence of steroids, whereas if it is formed a red color indicates the presence of terpenoids.

2.3. **Dry decrease**
Weigh the porcelain crucible that has been dried for 30 minutes in the oven at 105 °C. The extract was weighed as much as 1 gram and put into a porcelain crucible, then weighed. Then slowly shake the crush so that the extract is evenly distributed. Crush into the oven by opening the lid and leaving the lid in the oven. Crush containing the extract was heated at 105 °C for 1 hour. After that it is removed and cooled in a desiccator for 10-15 minutes then weigh it until a constant weight is obtained.

2.4. **Ash level**
The extract was weighed 2-3 grams, then put into a posselen crucible that had been glowing before. Crush is cooled in a desiccator and put in a fornes at 600 °C for 6 hours, so that the charcoal runs out which is marked with a gray color. After chilling, weigh it. Calculate the ash content (Depkes RI, 1995).

2.5. **Testing the antibacterial activity of ethanol extract, N-Hexane fraction, ethyl acetate and butanol of bitter melon against Staphylococcus epidermidis**
Prepare 9 test tubes then make the extract and fraction concentration of 80%; 40%; 20%; 10%; 5%; 2.5%; 1.25%; 0.625% and negative control. Then give labels with numbers 1 to 9. For a concentration of 80% (tube 1) weigh the extract/fraction 0.8 grams then dissolve it with DMSO until 1ml. For a concentration of 40% (tube 2) take 0.5 mL from tube 1 then add 0.5 mL of DMSO and so for the next concentration take 0.5 mL from the previous tube and then add 0.5 mL of DMSO then vortex each concentration. After that, make a *Staphylococcus epidermidis* bacterial suspension, by taking the *Staphylococcus epidermidis* bacteria using ose and inserting a tube that has been filled with
physiological NaCl then compare the turbidity of the suspension with 0.5 McFarland so that the density of the bacterial suspension is equivalent to an evenness of 0.5 McFarland.

Then prepare 9 test tubes again then fill with NB media as much as 0.8 mL in each tube and add a concentration of 0.1 mL of each concentration then add 0.1 mL of the Staphylococcus epidermidis bacterial suspension. For negative control, fill the test tube with 0.8 mL NB and add 0.1 mL of DMSO and then add 0.1 mL of Staphylococcus epidermidis bacterial suspension.

Next, vortex each tube, then incubate for 24 hours. Next do the planting on the MHA media, by preparing 9 sterile petri dishes and then filling them with 15 mL MHA media, let it solidify then spread 0.1 mL of NB media from each of the concentrations that have been incubated earlier and flatten using L stems. Each dilution was grown in duplicate and incubated at 37 °C for 24 hours. Then do the KBM observations by observing whether or not there are bacterial colonies growing on agar media.

3. Results and discussion

This research was conducted to determine the minimum bactericid concentration (MBC) of ethanol extract, n-hexane fraction, ethyl acetate and butanol of bitter melon (Momordica charantia L.) seeds against Staphylococcus epidermidis using the liquid dilution method, with a concentration of 80%; 40%; 20%; 10%; 5%; 2.5%; 1.25%; 0.625%. 1.5 kg of bitter melon seeds cleaned and then dried by aerating. The dried bitter melon seeds were blended into a fine powder as much as 820 grams with the aim of increasing the surface area of the sample so that the solvent could more easily enter the cells and attract the soluble active components out of the cell.

Extraction of the simplicial powder of bitter melon seeds was carried out using cold extraction, namely the maceration method. Put 820 grams of pare seeds simplicial into a black bottle using 70% ethanol as a solvent. Leave it for a few days while stirring occasionally until the color changes then filter then do the same treatment until you get a clear filtrate. The filtrate obtained is then evaporated with a rotary evaporator and the viscous extract is obtained as much as 125.4379 grams. So that the yield obtained is 15.2973%. Then the fractionation process was carried out using 75 grams of the thick extract obtained. This fractionation uses a different degree of polarity with a solvent, namely by using n-hexane as a nonpolar solvent, semi-polar ethyl acetate and butanol as a polar solvent. So that the yields of each were 5.4756% n-hexane, 2.5142% ethyl acetate and 8.09013% butanol. The drying shrinkage was obtained 8.74% at 105 ° C, the aim was to provide a maximum limit on the amount of compound lost in the drying process. The total ash content obtained is 2.28% the purpose of this ash content is to provide an overview of the mineral content obtained in the initial process until the extract is formed, where organic compounds and their derivatives are digested and evaporated so that only mineral elements and inorganic compounds remain [10].

After the phytochemical test was carried out, the ethanol extract of bitter melon seeds contained alkaloids, terpenoids and saponins. Whereas in the phytochemical test, the positive n-hexane fraction contained terpenoids because it formed a red color after being reacted with sulfuric acid H2SO4. Terpenoids have an antibacterial mechanism by destroying the bacterial cell membrane because triterpenoid compounds tend to be lipophilic. Cell membrane damage can occur when antibacterial active compounds react with the active side of the membrane or by dissolving lipid constituents and increasing their permeability. The bacterial cell membrane is composed of phospholipids and protein molecules. Due to increased permeability, cell membrane compounds or coagulation of the cytoplasm of these bacterial cells [11].

In testing of the ethyl acetate fraction, two secondary metabolites were found, namely alkaloids and terpenoids. Ethyl acetate is a semi-polar solvent that is able to attract compounds with a wide polarity range from polar to non-polar. Semi-polar solvents are able to extract phenolic compounds, terpenoids, alkaloids, glycons and glycosides. Meanwhile, non-polar solvents can extract chemical compounds such as wax, lipids and evaporating oil [10]. Alkaloids are difficult to dissolve in water, but dissolve in chloroform, ethyl acetate, acetone and alcohol. Alkaloids in plants are in their salt form so they are only soluble in inorganic solvents (chloroform, ethyl acetate, acetone, benzene, alcohol, ethanol and
methanol. The mechanism of action of alkaloids as antibacterials is by disrupting the peptidoglycan constituent components in bacterial cells. That the cell wall layer is not formed to benefit and causes cell death. Another alkaloid mechanism, namely alkaloid components, is known as DNA intercalator and inhibits bacterial cell typoisomerase enzymes [8]. Whereas in the phytochemical test on the butanol fraction, saponins were obtained which was indicated by the appearance of bubbles after strong shaking. The mechanism of action of saponins as antibacterials is to reduce surface tension resulting in increased permeability or cell leakage and resulting in intercellular compounds to diffuse through the cytoplasmic membrane so as to disrupt and reduce cell membrane stability. This causes the cytoplasm to leak from the cells resulting in cell death [8].

The antibacterial activity test in this study used liquid dilution. Performed by testing the antibacterial ethanol extract, n-hexane fraction, ethyl acetate and butanol of bitter melon seeds against Staphylococcus epidermidis, the concentration used was 80%; 40%; 20%; 10%; 5%; 2.5%; 1.25% and 0.625% and DMSO as negative control. By using NB as the medium. Each concentration contained 0.8 NB, 0.1 extract and 0.1 bacterial suspension, then vortexed and buried for 24 hours. Then to determine the value of KBM, planting was carried out on the media so that the concentration did not indicate the growth of bacterial colonies after incubation for 24 hours [12-15].

Table 1. Antibacterial test results of ethanol extract, n-hexane fraction, ethyl acetate and butanol in pare seeds.

| No | Concentration | Extract   | N-Hexane | Ethyl acetate | N-Butanol | Control |
|----|---------------|-----------|----------|---------------|-----------|---------|
| 1  | 80%           | Growth    | Growth   | Not Growth    | Growth    | Growth  |
| 2  | 40%           | Growth    | Growth   | Not Growth    | Growth    | Growth  |
| 3  | 20%           | Growth    | Growth   | Growth        | Growth    | Growth  |
| 4  | 10%           | Growth    | Growth   | Growth        | Growth    | Growth  |
| 5  | 5%            | Growth    | Growth   | Growth        | Growth    | Growth  |
| 6  | 2.5%          | Growth    | Growth   | Growth        | Growth    | Growth  |
| 7  | 1.25%         | Growth    | Growth   | Growth        | Growth    | Growth  |
| 8  | 0.625%        | Growth    | Growth   | Growth        | Growth    | Growth  |

To determine the value of the minimum bacterisid concentration (MBC) in the liquid dilution method, implantation of NB media that had been incubated for 24 hours into muller hinton agar (MHA) media was then observed for colony growth. The value of KBM is determined by looking at no bacterial growth at all in a petri dish that already contains agar media. In this test, only MBC was found in the ethyl acetate fraction, namely at a concentration of 80% and 40% there was no bacterial growth so that the MBC value of ethyl acetate was 40%. Meanwhile, the MBC for ethanol extract, n-hexane and butanol fraction occurred colony growth at each concentration so that the MBC value was not obtained.

4. Conclusion
The minimum bacterisid concentration (MBC) in this study was only obtained in the ethyl acetate fraction of bitter melon seeds, namely at a concentration of 40%, while in other extracts and fractions the value of MBC was not obtained because each concentration contained bacterial colony growth.

References
[1] Tan S P, Kha T C, Parks S E and Roach P D 2016 Food Rev. Int. 32 181
[2] Bai J, Zhu Y and Dong Y 2016 Ethnopharmacology 194 717
[3] Pujiyanto, Sunarmo and Ferniah R S 2017 Adv. Sci. Lett. 23 7
[4] Kuley E, Yazuver M N, Durmus N, Yazgan H, Gezginc Y, and Ozogul F 2019 Food Biosci. 32 100478
[5] Tinrat S and Asna M S 2016 Int. J. Pharm. Sci. 39 2
[6] Ngemenya M N, Mbah J A, Titanji V P K 2006 Afr. J. Tradit. Complement. Altern. Med. 3 2
[7] Nanasombat S, Kuncharoen N, Ritcharoon B, and Sukcharoen P 2018 Chiang Mai J. Sci. 45 1
[8] Coutinho H D M., Costa J G M, Falcão-Silva V S, Siqueira-Junior J P, and Lima E O 2010 Comp. Immunol. Microbiol. Infect. Dis. 33 6
[9] Roopashree T S, Dang R, Rani R H S, and Narendra C 2008 Int. J. Appl. Res. Nat. Prod. 1 3
[10] Kabir S R 2015 Appl. Biochem. Biotechnol. 175 5
[11] Hong L and Liping W 2010 Acta Hortic. 856 99
[12] Suparaja R and Usha R 2013 Int. J. Pharma Bio Sci. 4 1
[13] Balkhande S V and Surwase B S 2013 Asian J. Pharm. Clin. Res. 6 1
[14] Sajjan S, Chetana S H, Paarakh P M, and Vedamurthy A B 2010 Indian J. Nat. Prod. Resour. 1 3
[15] Kang J, Zeng B, Tang S, Wang M, Han X, Zhou C, Yan Q, He Z, Liu J, and Tan Z 2016 Asian-Australas. J. Anim. Sci. 29 4