UNDERGRADUATE THESIS

INHIBITION TEST OF SPRAY GEL WITH ETHANOLIC EXTRACTS FROM CHERRY LEAVES (*Muntingia calabura* L.) AGAINST *Staphylococcus aureus, Staphylococcus epidermidis* AND *Propionibacterium acnes*

by:

AISYAH RULINA SAFITRI

NIM 36.2015.7.1.2275

DEPARTEMENT OF PHARMACY

FACULTY OF HEALTH SCIENCES

UNIVERSITY OF DARUSSALAM GONTOR

2019

UNDERGRADUATE THESIS

INHIBITION TEST OF SPRAY GEL WITH ETHANOLIC EXTRACTS FROM CHERRY LEAVES (*Muntingia calabura* L.) AGAINST *Staphylococcus aureus, Staphylococcus epidermidis* AND *Propionibacterium acnes*

Submitted to Undergraduate Program University of Darussalam Gontor in Partticial Fulfillment of The Requirments for Health Science

AISYAH RULINA SAFITRI

NIM 36.2015.7.1.2275

Supervisor :

Nadia Saptarina, M.Farm, Apt

Solikah Ana Estikomah, S.Si, M.Si

DEPARTMENT OF PHARMACY

FACULTY OF HEALTH SCIENCES

UNIVERSITY OF DARUSSALAM GONTOR

PONOROGO

2019

UNIDA

GONTOR

UNIVERSITY OF DARUSSALAM GONTOR

ABSTRAK

UJI DAYA HAMBAT SEDIAAN GEL SEMPROT EKSTRAK ETANOL DAUN KERSEN (Muntingia calabura L.) TERHADAP *Staphylococcus aureus, Staphylococcus epidermidis* dan *Propionibacterium acnes*

Aisyah Rulina Safitri
36.2015.7.1.2275

Salah satu flora yang banyak ditemukan di Indonesia dan telah diuji kandungannya adalah tanaman kersen (*Muntingia calabura* L.). Tanaman ini memiliki senyawa metabolit sekunder yang dapat dimanfaatkan sebagai alternatif pengobatan. Potensi ini dimanfaatkan peneliti untuk membuat suatu formula antibakteri penyebab jerawat pengganti obat-obat antibakteri sintetis yang memiliki efek toksik obat dan diformulasikan sediaan gel semprot dengan zat aktif dari ekstrak etanol daun kersen. Tujuan dari penelitian ini untuk mengetahui aktivitas antibakteri sediaan gel semprot ekstrak etanol daun kersen terhadap *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), and *Propionibacterium acnes* (*P. acnes*).

Metode pada penelitian ini yaitu dibuat formula gel semprot dengan ekstrak daun kersen konsentrasi 10%. Uji aktivitas antibakteri yang digunakan adalah metode difusi padat dengan sumuran terhadap 3 bakteri penyebab jerawat, yaitu *S. aureus, S. epidermidis,* dan *P. acnes*. Kontrol positif yang digunakan adalah gel Klindamisin 1% dan kontrol negatif berupa basis gel semprot tanpa ekstrak. Diameter Zona Hambat (DZH) yang didapatkan, diolah menggunakan uji *Kruskal-walli*s dan uji lanjutan *Mann-whitney.*

Hasil dari analisis data adalah nilai sig. > nilai *p* (0,05%) yang mengartikan bahwa spray gel ekstrak etanol daun kersen dapat menghambat pertumbuhna bakteri *S. aureus, S. epidermidis,* and *P. acnes* dengan bukti terbentuknya zona bening di sekitar sumuran. Sediaan gel semprot ekstrak etanol daun kersen memiliki aktivitas antibakteri terhadap *S. aureus, S. epidermidis,* and *P. acnes* dengan kategori sedang. Tidak ada perbedaan pada tingkat penghambatan dari sediaan spray gel ekstrak etanol daun kersen terhadap *S. aureus, S. epidermidis*, and *P. acnes*.

Kata kunci: Uji daya hambat, *Muntingia calabura* L., gel semprot, Ekstrak etanol

ABSTRACT

INHIBITION TEST OF SPRAY GEL WITH ETHANOLIC EXTRACTS FROM CHERRY LEAVES (*Muntingia calabura* L.) AGAINST *Staphylococcus aureus, Staphylococcus epidermidis* AND *Propionibacterium acnes*

Aisyah Rulina Safitri
36.2015.7.1.2275

One of the flora that is found in Indonesia and has been tested for its contents is cherry (*Muntingia calabura* L.). This plant has secondary metabolites that can be used as an alternative treatment. This potential is used by the researcher to make an acne-causing antibacterial formula substitute for antibacterial synthesis drugs that have toxic drug effect then formulated spray gel preparations with active substances from the ethanol extract of *M. calabura* leaves. This research aims to know the antibacterial activity of spray gel preparation of ethanol extract of *M. calabura* leaves against *Staphylococcus aureus (S. aureus), Staphylococcus epidermidis (S. epidermidis)*, and *Propionibacterium acnes (P. acnes)*.

The method in this study is that the spray gel formulas are made with the extract of *M. calabura* leaves with concentration of 10%. Antibacterial activity testing was carried out by the diffusion method using wells on three acne-causing bacteria, namely *S. aureus, S. epidermidis,* and *P. acnes*. The positive control used was 1% clindamycin gel, and the negative control was a spray gel base without the extract. The diameter of the inhibition zone obtained was processed using the Kruskal-Wallis test and the Man-Whitney follow-up test.

The results from data analysis are sig. > *p* velue (0.05%) which showed that spray gel of ethanolic extract from cherry leaves can inhibit the growth of *S. aureus, S. epidermidis,* and *P. acnes* with the formation of a clear zone around the wells. The spray gel preparations of ethanol extract of cherry leaves have antibacterial activity against the *S. aureus, S. epidermidis* and *P. acnes* bacteria in the moderate category. Furthermore, there is no difference in the level of inhibition from spray gel preparations of ethanol extract of cherry leaves against *Staphylococcus aureus, Staphylococcus epidermidis* and *Propionibacterium acnes.*

Keywords: Inhibition test, *Muntingia calabura* L., spray gel, ethanol extracts

DECLARATION

I hereby

Name	: Aisyah Rulina Safitri
Student Number	: 36.2015.7.1.2275
Faculty	: Faculty Of Health Sciences
Department	: Pharmacy
Title	: Inhibition Test of Spray Gel with Ethanolic Extracts From Cherry Leaves (*Muntingia calabura* L.) Against *Staphylococcus aureus, Staphylococcus epidermidis* And *Propionibacterium acnes*

declare that this original thesis belongs to my work and does not to another researcher for a different degree. Furthermore, this thesis is never published before, except for some parts with their original references.

Otherwise, if it was found that this thesis contains plagiarism, I am ready to be ceased academically

Ngawi, 6th May, 2019

Author,

Aisyah Rulina Safitri
36.2015.7.2.1191

VALIDATION

INHIBITION TEST OF SPRAY GEL WITH ETHANOLIC EXTRACTS FROM CHERRY LEAF (*Muntingia calabura* L.) AGAINST *Staphylococcus aureus*, *Staphylococcus epidermidis* AND *Propionibacterium acnes*

Prepared and Presented by:

Aisyah Rulina Safitri
36.2015.7.1.2275

Has been approved by the Board of Examiners of Graduate Program

On May, 6th 2019

Board Examiners

Major Advisor

Nurul Marfu'ah, S.Si, M.Si

Examiner I

Nadia Saptarina, M.Farm, Apt

Examiner II

Solikah Ana Estikomah, S.Si, M.Si

This thesis is declared and accepted in fulfillment
To obtain a degree for Bachelor of Pharmacy
On May, 9th 2019

Head of Department of Pharmacy
Faculty of Health Science
University of Darussalam Gontor

Amal Fadholah, S.Si, M.Si, Apt

ACKNOWLEDGMENT

بسم الله الرحمن الرحيم

Assalamu'alaikum wa rahmatullahi wa barokatuh

Subhaanallah, thank God Almighty for all His blessings which have bestowed on all living things in the universe. Prayers and greetings that we always uphold for our Prophet, the Prophet Muhammad, who has channeled knowledge from Allah to all humanity.

Alhamdulillah, with the grace of Allah SWT the author can complete the writing of the thesis with the title " Inhibition Test of Spray Gel with Ethanolic Extracts From Cherry Leaves (*Muntingia calabura* L.) against *Staphylococcus aureus, Staphylococcus epidermidis* and *Propionibacterium acnes*", on time. This thesis was prepared as one of the requirements to obtain Bachelor's degree in Pharmacy at the Pharmacy Department at the Faculty of Health Sciences, University of Darussalam Gontor.

This thesis was conducted at the Pharmacy Microbiology Laboratory, University of Darussalam Gontor with the guidance and direction from the supervisors, laboratory staffs, and encouragement from various parties. Therefore, the author expresses her respect and gratitude to several parties, namely:

1. Both of the author's parents always give prayers, love, moral and material support to strengthen the author to finish this thesis on time.
2. *Al-Ustadz* Prof. Dr. KH. Amal Fathullah Zarkasyi, MA as the Rector of the University of Darussalam Gontor and his staff.
3. *Al-Ustadzah* drg. Hj. Ruskiyah Octavia, MM as Dean of the Faculty of Health Sciences, the University of Darussalam Gontor along with her staff.
4. *Al-Ustadz* Amal Fadholah, S.Si, M.Si, Apt as a Head of Pharmacy

Department of the University of Darussalam Gontor, as well as all lecturers and laboratory assistants for the guidance given during author's education in the Pharmacy Study Program at the Faculty of Health Sciences, University of Darussalam Gontor.

5. *Al-Ustadzah* Nadia Saptarina, M.Farm, Apt and *Al-Ustadzah* Solikah Ana Estikomah, M.Si as Advisors I and II, who are always patient to direct the author until the writing of this thesis is complete.
6. My little brothers Ahnaf Gilang Ramadhan and Ahmad Zufar Abdillah who faithfully entertained the author at the time of fatigue during the writing process.
7. Yayay as a friend of the research team who provided mutual assistance and support to each other.
8. The 2015 compatriots who always share stories, experiences, joys, help, and support so that the author can complete thesis writing and education in the Pharmacy Study Program at the Faculty of Health Science, University of Gontor.

he author realizes there are still many shortcomings and this thesis is still far from perfection. Therefore, the author expects constructive criticism and suggestions to complement the shortcomings of this study to make it better. Thus, hopefully that this thesis could be beneficial especially in the development of pharmaceutical science.

Wassalamu'alaikum wa rahmatullahi wa barokatuh

Ngawi, May 6th, 2019

Aisyah Rulina Safitri

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

CHAPTER I

INTRODUCTION

1.1. Background

Acne vulgaris acne is a skin disease chronic, obstructive and inflammation of the pilosebaceous unit that often occurs in adolescence. The prevalence of acne in adolescence is quite high, ranging between 47-90% during adolescence (Kurokawa et al., 2009). The disease is confined to the follicle polisebase heads and upper bodies because pilosebase glands in this area are very active. If pilosebase follicles are clogged, the sebum cannot get out and when collected in the follicle it becomes swollen, as well as there was the beginning of blackheads as a form of acne (Tranggono & Latifah, 2007). More over, other factors that cause blockage of the follicles are *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Propionibacterium acnes* are bacteria that cause acne (Ayu, 2009).

Acne treatment is given with antibiotics such as tetracycline, erythromycin, doxycycline, and clindamycin. The medication used benzoyl peroxide, azelaic acid, and retinoids (Oprica, 2004). The use of antibiotics for treating disease caused by a bacterial infection can lead to problems associated with the toxic effects of drugs, drug residues, and the development of resistant microbes. Judging from these problems, it is necessary that alternative medicine is more effective, efficient, and have minimal side effects (Monica et al., 2013).

Indonesia has many types of plants that can be used as traditional medicine (Miksusanti et al., 2009). Most people prefer their treatment options for medications from nature. Natural materials currently used in the treatment are more prevalent because the natural materials are considered to have lower side effects than synthetic drugs or chemicals, are more affordable and are easily available raw materials (Muhlisah, 2000). According to the resolution on Promoting the Role of Traditional Medicine

in Health Systems: Strategy for the African Region, about 80% of people in the member countries of the World Health Organization (WHO) in Africa already use traditional medicine for primary health purposes (DitjenPEN, 2014). Thus, the researcher must create an alternative treatment that can be used to treat diseases without the use of ingredients that are prohibited by Allah SWT. One alternative is to use herbal or natural materials. Natural materials are created by Allah SWT. it can be adapted to become the drugs of various diseases. Science all the diseases exist, God must have created a cure. As word of the Prophet Muhammad SAW:

مَا أَنْزَلَ اللهُ دَاءً إِلَّا أَنْزَلَ لَهُ شِفَاءً

> Meaning: «Allah has sent down a disease except lowering drugs for him» (HR. Ibnu Majah-3430).

The *Hadith* explains that God created all definite disease and its cure. Illness to humans comes from Allah, then Allah reason that He will provide a cure for the disease (Wulandari, 2017). God has created a whole spectrum of natural resources on earth for humans to use in their life, especially in medicine. The *Hadith* can be understood that as human beings, especially the scientists we have to take advantage of the abundant natural resources from God which can be used by the humans without having to use ingredients that are prohibited by the Creator of the disease.

Lots of efficacious natural ingredients have been formulated and have a positive effect on the inhibition of the growth of acne-causing bacteria, such as ginger rhizome (Fissy et al., 2014), narcissus bulbs (Yuni et al., 2013), star fruit (Ikhsanudin & Mardhiyah, 2017), *Excoecaria agallocha* leaves (Borman et al., 2015), soma leaves (Seli et al., 2015), paria (Laianto, 2014), lemongrass leaves (Sarlina et al., 2017), and cherry leaves (Handayani, 2014). The researcher chose cherry because these natural resources are very abundant and easy to obtain. Many natural materials will facilitate the production of large quantities.

Cherry (*Muntingia calabura* L.) is a tropical plant that is used as a shade plant. According to Sami et al. (2017), cherry leaves compounds are phenolic, flavonoids, and saponins which have strong antioxidant activity. According to a research by Putri (2016), cherry leaves extract can be used as an alternative to synthetic pesticides. Investigated also by Handy & Sentat (2016) on cherry leaves extract, it also has potential as a treatment for burns and also investigated by Heinrich et al. (2009) that the active ingredients in a cherry plant are flavonoid, sesquiterpenoid, and furan derivatives. Cherry leaves have many conscientious and effective as an anti-bacterial (Wulandari, 2017), (Handayani, 2014), (Arum et al., 2012). Other studies have also known that the cherry leaves have the potential to counteract the free radicals as exogenous antioxidants (Devi et al., 2016), (Sami et al., 2017), (Nurhasanah, 2012).

The gel dosage form is better to use in the treatment of acne because preparations of gel with a polar solvent is more easily cleaned from the surface of the skin after use and do not contain oils that can increase the severity of acne (Sasanti et al., 2006). One development in the treatment gel formulation is in the form of a spray (spray gel). This form has the advantage of another dosage form for delivering a substance into place without any irritation through contact with a cotton swab to minimize waste, reduce the possibility of contamination or infection and trauma to the patient. Furthermore, topical preparations by spray techniques are preferred over topical ointments or gels, especially for skin irritation (Jauregui et al., 2009).

In relation to the abundance in cherry leaves especially antibacterial activity, making researchers interested in testing the antibacterial effectiveness of the ethanol extract of cherry leaves (*Muntingia calabura* L.) which are formulated into a spray gel preparation against acne-causing bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Propionibacterium acnes.*

1.2. Formulation of the Problem

Based on the above background, the problem can be formulated as follows:

1. Can the spray gel preparation of ethanol extract of cherry leaves inhibit the growth of *Staphylococcus aureus, Staphylococcus epidermidis,* and *Propionibacterium acnes*?
2. How is the antibacterial activity from spray gel of ethanol extract from leaves of cherry (*Muntingia calabura* L.) against *Staphylococcus aureus*, *Staphylococcus epidermidis,* and *Propionibacterium acnes*?
3. Which bacteria has the most effective growth inhibited by the spray gel of ethanolic extract from cherry leaves?

1.3. Objectives of Research

1. To know whether the spray gel of ethanolic extract from cheery leaves preparation can inhibit the growth of *Staphylococcus aureus, Staphylococcus epidermidis*, and *Propionibacterium acnes*.
2. To Know the antibacterial activity from spray gel of ethanol extract of cherry leaves (*Muntingia calabura* L.) with a variety of compositions against *Staphylococcus aureus*, *Staphylococcus epidermidis,* and *Propionibacterium acnes*.
3. To find out which bacteria has the most effective growth inhibited by a gel of ethanolic extract from cherry leaves spray

1.4. Significance of Research

1.4.1. Theoretical Significance

The results of this study are expected to inform the public about the efficacy of the spray gel formulation containing ethanol extract of cherry leaves in inhibiting the growth of *Staphylococcus aureus*, *Staphylococcus epidermidis,* and *Propionibacterium acnes* as bacteria that cause acne.

1.4.2. Practical Significance

- The results of this study will be expected to add insight and knowledge in the field of health especially on the spray gel formulation with active ingredients from natural substances to inhibit the growth of *Staphylococcus aureus*, *Staphylococcus epidermidis,* and *Propionibacterium acnes.*
- To serve as the reference for the development of pharmaceutical preparations by spray gel system as an antimicrobial substance.

CHAPTER II

LITERATURE REVIEW

2.1. Previous Research

Cherry (*Muntingia calabura* L.) is a plant that is extremely easy to grow in a variety of environments without the need to use special care in its growth. With the abundance of natural resources, many researchers are interested in knowing more about their contents so that they can be utilized especially in the field of medicine. Arum et al. (2012) stated that in cherry leaf simplicia powder there is a phytochemical content in the form of flavonoids, triterpenoids, saponins, and steroids. The cherry leaves are then extracted using methanol and ethanol solvents and then tested the antibacterial activity against *E. coli, P. aeruginosa, S. aureus*, and *B. subtilis* bacteria. The results showed that cherry leaves with ethanol and methanol extract had the antibacterial activity are tested against test bacteria in concentrations of 96%, 75%, and 50%. In the study of Buhian et al. (2016) it also found that in cherry leaves there is phytochemical content in the form of sterols, flavonoids, alkaloids, saponins, glycosides, and tannins. After that, an antibiotic test was conducted on *E. coli, S. thypi, P. aurugosa, S. aureus, B. subtilis,* and *C. albicans* fungi. The result is that the cherry leaves with ethanol extract is effective as an antibiotic of *C. albicans, S. aureus,* and *P. aeruginosa.*

More over, it is examined by Handayani (2014) on antibacterial activity against *S. epidermidis* bacteria. Ethanol extract of cherry leaves was made into various concentrations of 1ppm, 3ppm, 5ppm, and 9ppm. The results show that the ethanol extract of cherry leaves has the ability to inhibit the growth of *Staphylococcus epidermidis* at the concentrations of 3ppm, 5ppm, and 9ppm with diameter of inhibition zone 10.30 mm, 11.27 mm, 14 mm respectively. A study by Apriliyanti (2016) is also conducted on the antibacterial activity against *P.acnes* bacteria in concentrations of 10,

20, 30, 40, 50, 60, 70, 80, 90, 100%. The lowest inhibition zone results were from a concentration of 10%, namely 12 mm, and the highest concentration of 100% was 33 mm. From testing these extracts, many studies have begun to formulate them in various dosage forms so that they are easy to use. Wulandari (2017) conducted a study by formulating cherry leaf extract with concentrations of 5%, 10%, and 15% into microemulsion preparations. The results showed good characteristics with 10% cherry leaves extract concentration. In addition, it can inhibit the growth of *Staphylococcus epidermidis* with the antibacterial activity of 8.34 which is categorized as moderate.

2.2. Literature Review

2.2.1. Spray Gel Dosage Form

Cosmetic products are referred to as a gel are usually in the form of semisolid and are clear to opaque (translucent). Jelly is also called "gel", is a semisolid system consisting of a suspension made of small inorganic particles or large organic molecules, penetrated by a liquid. If the mass of the gel consists of a network of separate small particles, the gel can be classified as a two-phase system (Ditjen POM, 2014). The range of viscosity ranges from pourable viscous liquid to soft solid rod. The gel product is a product in which the flow properties (rheology) have modified and made liquefying shear (shear thinning), compared to the flow properties of non-Newtonian (Agoes, 2015).

Single-phase gels and jellies can be described as a three-dimensional network formed by the addition of macromolecules such as proteins, polysaccharides, and synthetic macromolecules in the appropriate liquid (water and solvent in pharmaceutical hydroalcoholic). Many polymer gels show reversibility between gel and sol state which is a liquid phase containing macromolecules dispersed or dissolved. Only the formation of several polymer gels is irreversible because its chains are covalently bonded. The 3-dimensional network formed

by the two-phase gel and jelly are formed by some of the colloidal inorganic clay (Agoes, Sediaan Farmasi Likuida-Semisolida (SFI-7), 2012).

Single-phase gels which consis organic macromolecules distributed uniform in a liquid in such that there is no visible bond between the macromolecular dispersed and fluid. Single-phase gels may be made from synthetic macromolecules (e.g., carbomer) or natural gums (e.g., tragacanth) even though gels generally consist of water, ethanol and oil can use as a carrier phase (Ditjen POM, 2014).

According to Agoes (2015), water gel products also used as skin moisturizer products. The main function of gelling agents is to create a product which contains more than 90% water to be easier to handle and produce aesthetically appealing appearance when applied to the skin. The viscosity of the gel structure also helps the suspension of other formulation components such as pigments, large oil droplets, or particulate particles. Some properties of skin care products also apply to hair care products (Agoes, 2015). A good gel viscosity is at a pH of 6-11 while at pH less than 3 with more than 12 gel the viscosity decreases (Lachman et al., 1994). The gel formulation was chosen as the treatment of acne because it contains no oil preparations that can increase the severity of acne (Borman et al., 2015).

2.2.2. Cherry Plant

Cherry plant (*Muntingia calabura* L.) has another name as Cherry (Jakarta), Baleci (Madura), Ceri (Java). The tree can reach a height of up to 12 meters with horizontal branches and form a shady shade. The leaf lies flat and alternate while leaf blade is not symmetrical, oval, serrated edges and a pointed end, the bottom surface is dense, grey-haired, and short-stemmed. The flowers are in a bouquet, composed of 3-5 flowers, located in the axillary, long-stemmed, petal share in, flat-brimmed crown, obovate, white, thin, and bald. Long-stemmed Berry, almost perfectly round, 1-1.5 cm in diameter, yellow-green and turns

red when ripe (Hidayat & Napitupulu, 2015). According to Steenis et al. (2005), the classification of the cherry plant is as follows:

Kingdom : Plantae
Superdivision : Angiospermae
Class : Dialypetalae
Ordo : Malvales
Family : Tiliaceae
Genus : Muntingia
Species : *Muntingia calabura L.*

Figure 1. Leaf cherry (Muntingia calabura L.)

There are many benefits contained in the cherry leaves. Some previous researchers have researched the efficacy of this plant namely as a drug cough, sputum (Hutapea, 1994), antitumor, antibacterial, antioxidant, antiproliferative, antihyperglycemic, antiseptic and can overcome the blood sugar disease. Cherry leaves are conscientious and effective anti-bacterial (Wulandari, 2017), (Handayani, 2014), (Arum et al., 2012). According to a research by Ramasamy et al., 2017) the methanol extract of *Muntingia calabura* L. was effective against *Xanthomonas campestris* pv. *Oryzae, Erwinia amyovora*, and *Agrobacterium tumefaciens*. Another study stated that the fractions isolated from the ethyl acetate fractions of *M. calabura* methanol extract were effective as an antibacterial against *S. aureus* 25923 and *S. aureus* 33591 (Zakaria et al., 2010). Another study also mentions that

the cherry leaves have a potentials as an exogenous antioxidants (Devi et al., 2016), (Sami et al., 2017), lowering cholesterol levels (Putri et al., 2018), the healing of burns (Handayani & Sentat, 2016), etc.

According to Ratnasari (2017), the phytochemical content of cherry leaves extract (*Muntingia calabura* L.) with ethanol and methanol are alkaloids, flavonoids, saponins, tannins, and steroids. According to Buhian *et al*. (2016), cherry leaves contain antibacterial against several microbes, namely:

Organisms	Average inhibition zone diameter (mm)		MIC (mg/mL)	
	Leaf extract	Stem extract	Leaf extract	Stem extract
E. coli	12.3	10.0	–	–
P. aeruginosa	20.0	15.7	2.500	2.500
S. typhimurium	19.0	19.0	> 10.000	> 10.000
S. aureus	37.7	24.7	1.250	1.250
B. subtilis	17.0	16.0	> 10.000	> 10.000
C. albicans	18.7	19.0	0.625[a]	2.500

[a]: MIC may be 0.625 mg/mL or lower.

Figure 2. Size of inhibition zone of cherry leaves extract on various microorganisms

The biological chemicals contained in cherry leaves are:

1. Flavonoids

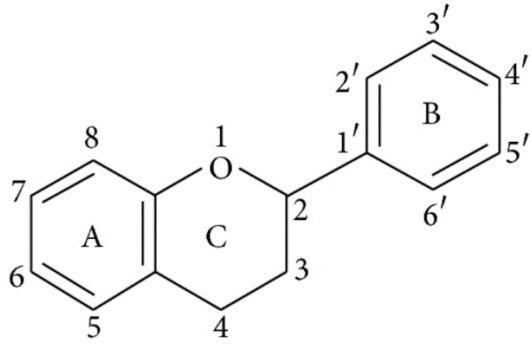

Figure 3. Flavonoid compounds structure (Pandey, 2013).

Flavonoids according to the parent compound structure is derived from flavonoids contained in the form of white powder on primroses plants in which all of them have saveral common traits. There are about ten classes known as flavonoids such as anthocyanins, proanthocyanin, flavonols, flavones, glikoflavon, biflavon, khalkon and auron, flavanones, and isoflavones (Harborne, 1987). Flavonoids are found in all parts of the plant including the fruit, pollen, and roots (Sirait, 2007).

Flavonoids are the form of water-soluble compounds. They can be extracted with ethanol 70% and remain in the water layer after the extract was shaken with petroleum ether. Flavonoids are phenolic compounds in which the color changes when added base or ammonia, so it can be easily detected on the chromatogram or in solution (Harborne, 1987). Generally, the flavonoid is found to bind to the sugars to form glycosides that cause these compounds to be more soluble in polar solvents, such as methanol, ethanol, butanol and ethyl acetate (Hanani, 2015). Flavonoids have antibacterial activity by forming a complex of proteins and denature bacterial cell protein so that the bacterial cell membrane becomes damaged and cannot be repaired anymore (Juliantina et al., 2008).

2. Tanin

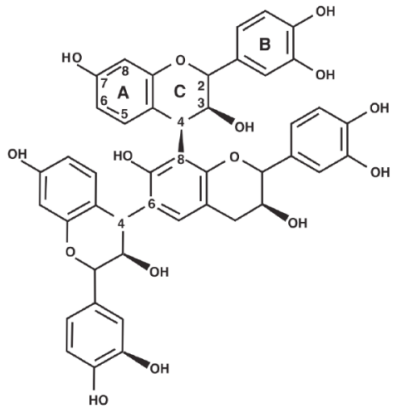

Figure 4. Tannin compound structure (Lambert, 2013).

Tannins are broadly in the vascular plant, in angiosperms are specialized in timber network (Harborne, 1987). Tannins are composed of water-soluble polyphenol compounds which can have a high molecular weight (Heinrich et al., 2009). Astringent properties of tannins can be used as antidiarrheal to stop the bleeding and prevent inflammation especially in the oral mucosa, as well as used as an antidote to the heavy metals and alkaloids poisoning (Hanani, 2015). Tannins also have antibacterial activity by having the ability to form complex compounds with bacterial cell protein through hydrogen bonds. If the hydrogen bonds formed between tannin with protein, the protein will be denatured so that the bacterial metabolism becomes impaired and results in bacterial cell death (Ajizah, 2004).

3. Saponin

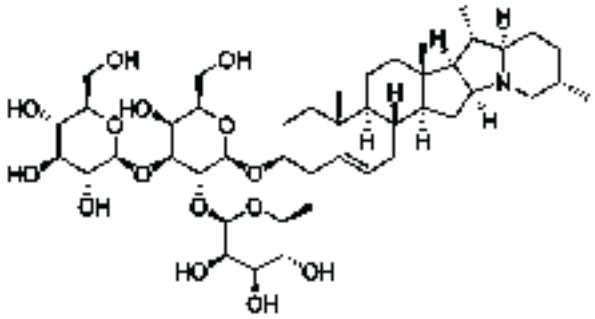

Figure 5. Saponin compound structure (Burcio, 2017).

Saponins are widespread in plants and can also be called triterpene glycosides. Its soap character are similar to forming a foam (Heinrich et al., 2009). Saponins can be used as antibacterial in a manner which react with porin transmembrane protein constituent polymer forming strong bonds that can damage porin. If porin as a doorway compound is damaged, it can reduce the permeability of the cell membrane of bacteria so that the bacterial cells are lack of nutrition. As a result, the bacterial growth is inhibited and dead (Utami, 2008),

4. Alkaloid

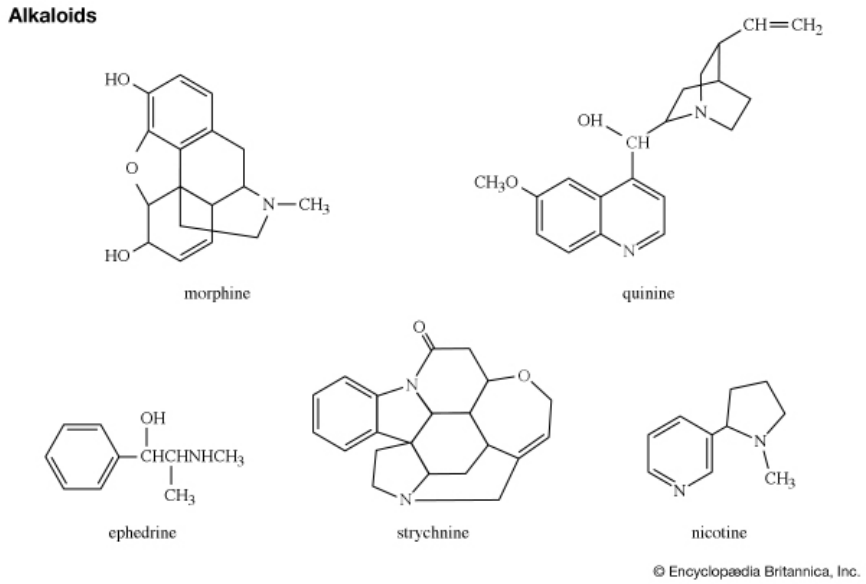

Figure 6. Alkaloids compound stucture (Encyclopaedia Britannica, 2018).

Alkaloids are secondary metabolites of compounds containing the element nitrogen (N) normally on the ring of heterocycles and alkaline. Alkaloids in plants are generally in the form of salt that binds to organic acids contained in the plant such as succinic acid, maleic, mekonat, kinat, and is soluble in polar solvents ethanol or water. Functions such as alkaloids in plants are to defend themselves against microorganisms, viruses or insects, and as a plant growth regulator (Hanani, 2015).

This secondary metabolic is most commonly used in the medical and pharmaceutical world. Plants and fungi which are rich in this material are widely used to relieve pain and as a recreational stimulant. German pharmacist Friedrich Wilhelm Karl Meissner first coined the term ‘alkaloid’ in 1818 to describe a compound that has alkaline properties (so-called alkaloids) (Heinrich et al., 2009).

Alkaloids possess antibacterial activity by the way of interfering

with the components of peptidoglycan in the bacterial cell, so the cell wall layers are not fully formed and cause bacterial cell to die. In addition, alkaloid compounds contain basic nitrogen groups which when reacted with amino acid compounds in the cell wall of bacteria it may result in changes in the amino acid structure. As a result, the genetic changes in the DNA chain balance are damaged and lyse the bacterial cells that cause bacterial cell to die (Juliantina et al., 2008).

2.2.3 Extraction

Extraction is the process of separating two or more substances with solvent which are not mutually interfered, either from a liquid to a liquid or of solids into liquids. Extraction is usually done to isolate a compound nature of the original network of herbs and dried (Kusnaeni, 2008). The purpose of the extraction is to attract or separate the compounds of a mixture or simplicia (Hanani, 2015).

Extracts are concentrated preparations obtained by extracting active substance of simplicia vegetable or animal crude drugs using a suitable solvent, then all or almost all of the solvent is evaporated and remains the powder mass or treated in that way to meet the standard that has been set (Ditjen POM, 2014).

Selection of solvent extraction is extremely important. Failure in extracting biomass could lead to losing access to obtain the desired active substance. In addition, the use of improper extraction methods such as strong heating of the biomass with a solvent can cause decomposition of natural ingredients that results in lost biological activity (Heinrich et al., 2009). Solvents used in the extraction process is ethanol cherry leaves. Ethanol is a universal solvent that easily dissolves alcohol as a class of compounds which correspond to low enough so it can be easily evaporated without using high temperatures, inert, and has a reasonable price. In addition, ethanol is the smallest solvent in its toxicity compared to other alcohols that have a value of LC50 7060 mg/kg (Guenther, 2006).

Maceration is a crude drug extraction by soaking a solvent at room temperature so that the destruction or degradation of metabolites can be minimized. In the maceration, a process of concentration balance is between solvent outside and inside the cell so that the necessary replacement of solvent happens repeatedly. The main advantage of maceration extraction methods is that the procedures and equipment used are not heated, simple, and the natural materials do not become loose. Cold extraction allows many compounds extracted although several compounds have limited solubility in the solvent at room temperature (Hanani, 2015).

Evaporation (concentration/thickening) extraction results which still contain many solvent are intended to obtain a more concentrated extract to keep the concentration of the compound larger and easy to store (Hanani, 2015). Thickening generally uses the oven and bulk freeze dryer (Saifudin et al., 2011). In the process of concentration, the temperature should not be too high to prevent the decomposition of the compounds in the extract.

2.2.4. Acne

The outer portion of the skin is the epidermis. This section is composed of stratified squamous epithelial tissue keratinized. This network has no blood vessels and the cells are extremely tightly. The thickest part of the epidermis can be found on the palms and soles are experiencing are stratified into five layers of the skin: basal stratum, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum (Agoes, 2015). The most important function of the skin is as a protective (barrier) between the individual and the surrounding environment. This barrier must be passed by the parasite when about to go into the internal environment of an individual. The barrier will be broken in case of penetrating injuries and body content flows out (Underwood, 2000).

Acne is a skin disease caused by chronic inflammation of the gland polisebasea marked by numerous blackheads, papules, pustules, and cysts nodes predilection. Microorganisms such as *Staphylococcus epidermidis* and *Propionibacterium acnes* are involved in the pathogenesis of this disease by producing metabolites that can react with the sebum thus increasing the inflammatory process (Tranggono & Latifah, 2007). The pathogenesis of acne includes four factors namely hyperpoliferation follicular epidermis, excessive sebum production, inflammation, and the activity of *P.acnes* (Movita, 2013).

The degree of acne based on type and number of lesions can classify as mild, moderate, heavy and extremely heavy (Movita, 2013).

Table 1. The degree of acne severity

Level	Blackheads	Papule	Nodule	Inflammation	Scar tissue
Light	<10	<10	-	-	-
Moderate	<20	<10-50	-	+	±
Heavy	>20-50	>50-100	<5	++	++
Very heavy	>50	>100	>5	+++	+++

(-) none, (±) can be found, (+) there are, (++) quite a lot, (+++) a lot

The goal of therapy is to correct acne follicle keratinization, decrease sebaceous gland activity, reduce the population of *P.acnes* bacteria and reduce inflammation (Movita, 2013). Treatment of acne and related conditions improve cosmetics and self-image patients and prevent scarring associated with acne (Price & Lorraine, 2006). Acne treatment can also be determined by determining the degree of acne. Most mild to moderate acne requires topical therapy. Moderate to severe acne can use a combination of topical and oral therapies (Cunliff et al., 2001).

2.2.5. Staphylococcus aureus

Staphylococcus aureus once regarded as the only pathogen of the genus. *Staphylococcus aureus* carrier are asymptomatic and often found in which this organism is found in 40% of healthy people specifically in the nose, skin, armpits, or perineum (Irianto, 2013). *Staphylococcus aureus* is positive coagulase (instead of the normal flora of humans) which is a major pathogen for humans (Brook et al., 2005). *Staphylococcus aureus* is a Gram-positive spherical bacterial with the diameter of 0,7 to 1,2 μm, are arranged in irregular groups such as grapes, facultatively anaerobic, spore-forming, and does not move (Jewetz & Adelberg's, 2010).

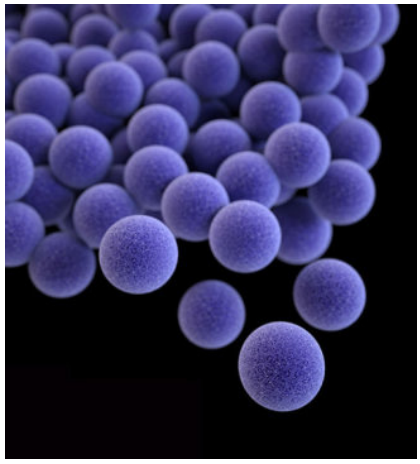

Figure 7. Staphylococcus aureus (Lutz, 2015).

Classification of *Staphylococcus aureus*, according to Dwidjoseputro (1998) are:

Kingdom : Procaryota
Division : Firmicutes
Class : Bacilli
Ordo : Bacillales
Family : Staphylococcaceae
Genus : Staphylococcus
Species : *Staphylococcus aureus*

Staphylococcus aureus causes a broad range of infectious syndrome. Skin infections can occur in warm humid conditions or when skin as exposed (Irianto, 2013). Some diseases caused by *Staphylococcus aureus* are boils, acne, impetigo, and wound infections. *Staphylococcus aureus* infection is characterized by tissue damage accompanied by pus abscess (Warsa, 1994).

2.2.6. Staphylococcus epidermidis

Staphylococcus epidermidis is gram-positive, aerobic or facultative anaerobes. It is a normal human flora, generally found on the skin flora, and few in the mucosal flora. These bacteria also cause the release of oleic acid in which the result of hydrolysis by lipase is supposed to influence the development of (Saising et al., 2008). Staphylococcus are usually arranged in irregular groups like wine. This organism is easy to grow in many types of medium, metabolically active, ferment carbohydrates and produce the pigment that varies from white to dark yellow (Jewetz & Adelberg's, 2010).

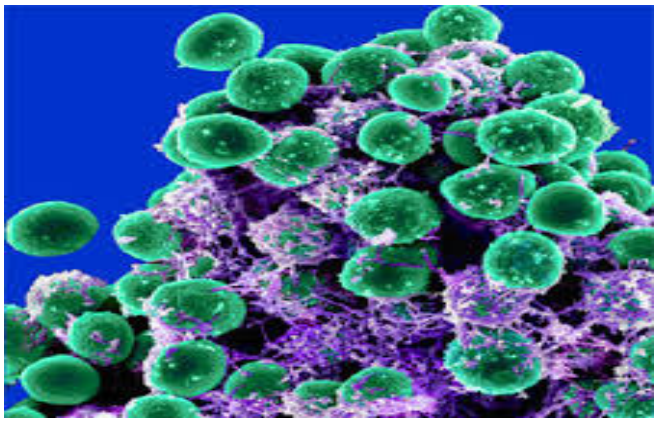

Figure 8. Staphylococcus epidermidis (NIAID, 2011).

Classification of *Staphylococcus epidermidis*, according to Jewetz & Adelberg's (2010) are as follows:

Division : Eukaryotes
Class : Schizomycetes

Ordo : Eubacteriales
Family : Micrococcaceae
Genus : Staphylococcus
Species : *Staphylococcus epidermidis.*

Bacteria that have this Staphylococcus genus have morphological characteristics such as colony color is milky white or slightly creamy, rounded colony shape, raised edge, as well as cell shape of a ball, a diameter of 0.5-1.5 μm and facultatively anaerobic. *Staphylococcus epidermidis* can cause minor skin infections accompanied by abscess formation. *Staphylococcus epidermidis* biotype-1 can cause chronic infections in humans (Radji, 2011).

The role of *Staphylococcus epidermidis* in the pathogenesis of acne which has a lipase enzyme can hydrolyze triglycerides in the sebaceous unit into free fatty acids that can lead to keratinization and inflammation that cause acne (Kligman, 1994)**.** Generally, these bacteria can cause swelling (abscess) such as acne, skin infections, urinary tract infections, and kidney infections (Radji, 2011). Besides, these bacteria can also cause infections in neonates people whose immune system is low, and in patients who use the device mounted in the body (Hart & Shears, 2004).

2.2.7. Propionibacterium acnes

Propionibacterium species are normal flora of the skin, mouth, colon, conjunctiva, and salutary outer ear. Propionate acid metabolic product form to be the origin of the name of this genus. In the Gram stain, the species is highly pleomorphic, showing a curved tip, shaped mace or pointed, has long shaped with uneven coloring such as beads, and sometimes shaped or spherical kokoid (Jewetz & Adelberg's, 2010). *Propionibacterium acne* are bacteria that grow relatively slowly. These bacteria are anaerobic positive gram strain bacteria typically tolerant of air. The genome of this bacterium has assembled and a study shows that some genes to produce the enzyme to remove the skin and proteins

which may be immunogenic (activating the immune system) (Brook et al., 2005).

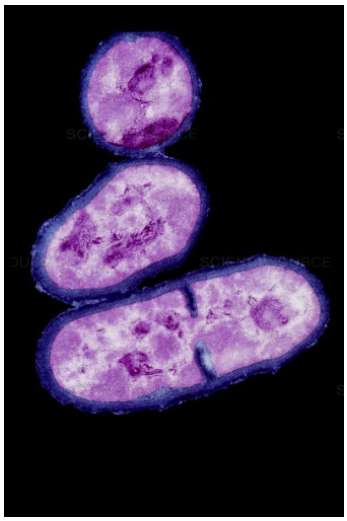

Figure 9. Propionibacterium acnes (Kwangshin, 2015).

The classification of *Propionibacterium acnes* according to Waluyo (2007) as follows:

Kingdom : Bacteria
Phylum : Actinobacteria
Class : Actinobacteridae
Ordo : Actinomycetales
Family : Propionibacteriaceae
Genus : Propionibacterium
Species : *Propionibacterium acnes*.

The essential features of the *Propionibacterium acnes* bacterium is a irregular rod-shaped seen in a positive gram stain. These bacteria can grow in the air and do not produce endospores. These bacteria can form branched filaments or a mixture in the form of rods/filaments to form kokoid. *Propionibacterium acnes* require oxygen from aerobic or facultative anaerobes to mikroerofilik or anaerobic. Mean while, some are pathogenic for animals and plants (Waluyo, 2007),

Propionibacterium acnes is often regarded as opportunistic pathogens, causing diseases of acne vulgaris and is associated with a variety of inflammatory conditions. This causes acne bacteria to produce lipase which liberates free fatty acids from the fat in the skin. These fatty acids can cause any tissue inflammation which plays a role in the onset of acne. In addition, *P.acnes* is a frequent cause of postoperative wound infections especially in surgery involving the installation of equipment such as infection in prosthetic joints. Because it is part of normal skin flora, *P.acnes* typically contaminates the blood or cerebrospinal fluid cultures taken with the penetration of the skin (Jewetz & Adelberg, 2012).

2.2.8. Antibacterial Activity Test

According to Harti (2015), several methods in the antibacterial activity test are as follows:

1) Diffusion method

   This method is usually referred to as disc-diffusion method or the Kirby-Bauer test. A disc is placed in antibacterial surface inoculated agar medium in alignment, incubated and observed the formation of inhibition zone. This method is used to determine the MIC (Minimum Inhibitory Concentration) on the lowest concentration of an antibacterial that could inhibit bacterial growth visually. The disadvantage it is unable to determine the bactericidal effect of an antibiotic. Other diffusion methods are E-test and ditch-plate technique.

2) Dilution method

   This method is used to determine the MIC / MBC and also to know the MKC (Minimum Killing Concentration. Inoculated with a dilution series of antibacterial, a tube containing a liquid medium and inoculated with the test the bacteria was observed its turbidity level/growth. This method is divided into two kinds, which are agar dilution and broth dilution.

2.2.9. Halal product analysis

The word *halal (halāl, halaal)* is an Arabic term in Islam which means "allowed". Etymologically, *halal* means things that are permissible and can be done because they are free or not bound by the provisions that prohibit it (Qardhawi, 2007). Halal terms in everyday life are often used for foods or drinks obtained for consumption according to Islamic law.

According to the Indonesian Ulema Council (MUI), a product is said to be *halal* if the product is aallowed by the *Shari'a* of Islam. The meaning of halal products is products that fulfill halal requirements by Islamic law (Depag, 2003). Terms of halal products include:

a. Does not contain pork and ingredients derived from pigs.
b. Does not contain prohibited ingredients such as materials derived from human organisms, blood, dirt, and others
c. All ingredients are derived from halal animals are slaughtered according to procedures of Islamic law.
d. All storage, sales, processing, premises management, and transportation should not be used for pigs. If it is once used for pigs or other non-halal items, it must first be cleaned in a manner that is arranged according to Islamic law.
e. All foods and drinks that do not contain *khamar.*

In summary, the terms of *halal* products according to Islam are *halal* substances, *halal* ways to obtain them, *halal* in the process, *halal* in its storage, *halal* in its transportation and *halal* in the presentation.

Products used by consumers especially Muslims must use products that must provide good benefits, do not cause harm or do not harm consumers in the form of health or morals. Thus, consumers can get the maximum benefit with provisions of Islamic teachings.

2.3. Conceptual framework

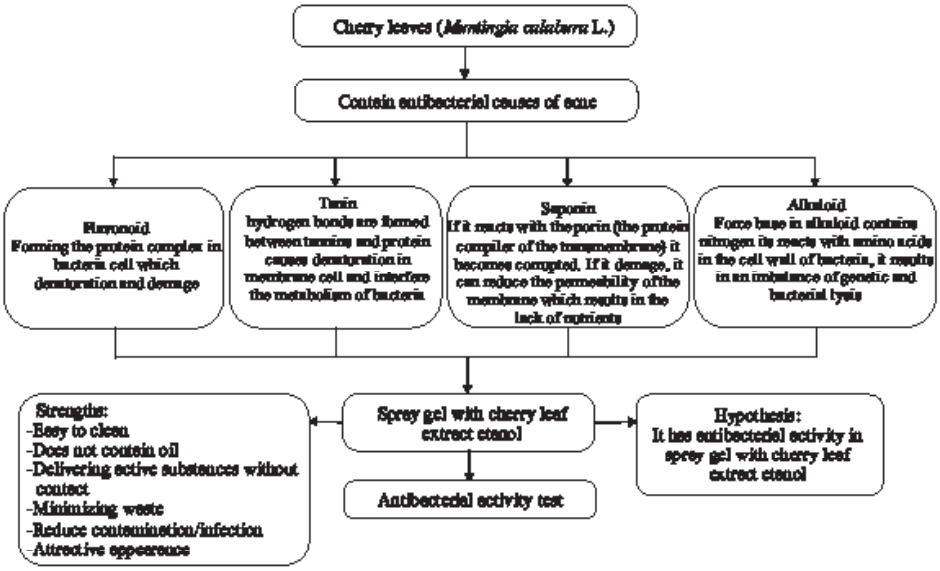

Figure 10. Conceptual framework scheme

2.4. Hypothesis

From this research, it can be hypothesized that there was the inhibition activity in spray gel preparation of ethanol extracts from cherry leaves (*Muntingia calabura* L.) against acne-causing bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis,* and *Propionibacterium acnes*.

CHAPTER III

RESEARCH METHOD

3.1. Research Setting

This research was conducted in the Laboratory of Microbiology Pharmacy, University of Darussalam Gontor, Mantingan, Ngawi, East Java. The research period started from October 2018 until January 2019.

3.2. Materials and Instrument

Samples of plants used are the cherry leaves (*Muntingia calabura* L.) which are not too young or too old obtained from the University Darussalam Gontor area, Mantingan, Ngawi, East Java. The solvents used in the extraction process is 70% ethanol. The composition of the spray gel of ethanolic extract from cherry leaves are as follows:

Table 2. Spray Gel Composition Preparation

Material composition	Function	Total (%)
Extract of *Muntingia calabura* L.	Antibacterial substances	10
Carbopol 940	Gelling agent	1.5
Poloxamer 407	Filming agent	0.1
Glycerin	Humectant	1
NaOH	Dispersion neutralizing carbopol	0.20
Dinatrium edetate	Chelating agents	0.1
NaCl	Viscosity regulators	1
Matrium benzoate	Preservative	0.2
Add Aquadest	Solvent	Ad 100

The materials used in antibacterial testing was 0.9% NaCl, Nutrient Agar media, clindamycin gel, Tween 80, distilled water and 70% ethanol. The tools used in this study include: analytical balance, blender, macerator, vacuum rotary evaporator, aluminum foil, incubators, laminar air flow cabinet, autoclave, vortex, magnetic stirrer, hotplate, plastic wrap,

micropipette, loopful small round , cotton, ovens, refrigerator, water bath sonicator, spray bottles, containers of glass, mica, paper label and glass tools commonly used in laboratories such as beaker glass, funnel, watch glass, spatula, stir bar, a pipette.

3.3. Research Design

This study was an experimental study to observe the spray gel preparation of antibacterial activity of ethanol extract of cherry leaves against the three bacteria that cause acne which are *Staphylococcus aureus, Staphylococcus epidermidis* and *Propionibacterium acnes*. The study design used was Completely Randomized Design (CRD). The method used to determine the antibacterial activity of spray gel against three bacteria is diffusion method.

Based on the method of CRD, there were three different samples (F, K +, K-) against three kinds of bacteria with three repetitions per treatment. The variables used in this study are two, namely the independent variable and the dependent variable. The independent variable is in the form of spray gel with a cherry leaf ethanol extracts namely F. Then, the dependent variable it in the form of inhibition growth of three bacteria that cause acne, namely *Staphylococcus aureus, Staphylococcus epidermidis,* and *Propionibacterium acnes*. It created a positive control in the form of clindamycin 1% gel (K+) and negative controls in the form of spray gel without ethanol extract of leaves of cherry (K-). Then, it measured the inhibition zone formed after being treated.

3.4. Research Procedure

3.4.1. Plants Determination

The leaves were obtained from the University Darussalam Gontor area, Mantingan, Ngawi, East Java it is determined at the Laboratory of Pharmaceutical Biology, Faculty of Pharmacy, University of Gadjah Mada, Yogyakarta. For the results, see Appendix 1.

3.4.2. Preparation and Sterilization Equipment

The tools used in the study of antibacterial activity are sterilized beforehand. Tools such as glass beaker, measuring cup, flask and rubber pipette have been wrapped and sterilized in an autoclave at a temperature of 121^{o} C for 15 minutes. Tools such as stirring rod, tweezers, spatula, watch glass-wrapped are put in an oven at a temperature of 160-170^{o} C for ± 2 hours. Ose needle and tweezers are burned with the fire burning over a bunsen.

3.4.3. Extraction process

Preparation of extracts are made by maceration. This process begins with the selection of cherry leaves according to the criteria. Among others, the criteria are the leaves should be flat, symmetrical leaf blade, serrated edge, sharp edge, the leaves taken are the ones located on 3, 4, 5 number of shoots (Purwonegoro, 1997). Furthermore, wash the leaves with water flowing and clean them then put them into the oven with a temperature of 60° C for 24 hours (Alkhakim et al., 2013). The leaves are mashed with a grinding machine into powder and sieved using a sieve size of 44 mesh. Cherry leaves powder produced are weighed and taken as many as 500 g. 500 g of cherry leaf powder are inserted into the container maceration and soaked in 70% ethanol at a ratio of 1:2 (W:V) (Apriliyanti, 2016). The researcher prefer to choose 70% ethanol as a solvent because it is selective and easily mixed with water with all comparisons. The use of 70% ethanol with solvents can attract huge chemical compound contained in simplicia.In addition, 70% of ethanol has advantages compared to other solvents because of the low level of toxicity and it is more economical (Aziz, 2010).

Maceration is closed in the container and stored for 1x24 hours in a place without sun exposure. While stirring, then filtered, separated between the pulp and the filtrate. Pulp is extracted again with 70% ethanol for 2 x 24 hours. The filtrate obtained is then collected, concentrated and evaporated with a rotary evaporator at a temperature

of 40° C ± 4 hours to obtain a viscous ethanol extract. Then, they are concentrated again using a water-bath for 2 x 24 hours until the extract becomes thick (Tamu, 2017).

Furthermore, the extract obtained was tested for its characteristics including physical characteristics, calculation of extract yield and calculation of water content extract. The standard water content allowed in thick extracts is not more than 10% (Depkes RI, 1986). The less water content in the extract can reduce the possibility of extracts to be contaminated by microorganisms (Saifudin et al., 2011). In addition, the small water content in the extract can reduce enzymatic processes that can change the useful chemical content into other products that no longer have pharmacological effects (Ma'mun et al., 2006).

3.4.4. Phytochemical Screening

The chemical content contained in the extract is tested qualitatively, including:

a. Identification of flavonoid compounds

   An extract weighing 0.1 g is placed on the watch glass. Furthermore, it was dripped with NaOH solution with a concentration of 10% in 2 drops. If there is a change in color to yellow or orange, then the extract is predicted to positively contain flavonoids (Pakaya et al., 2015)

b. Identification of alkaloid compounds

   A thick extract is taken as much as 0.1 g, and HCl solution was added with a concentration of 2 mL and then shaken. Then put one drop of Wagner reagent and see the color change. The extract is said to be positive for alkaloids if, after testing, brown or red deposits formed (Tiwari et al., 2011).

c. Identification of saponin compounds

   Weigh the thick extract by 0.1 g and place it in a test tube. Then put 10 ml of aquadest and shake hard in 1 minute. The extract

positively contains saponins with foam formation (Hanani, 2015).

d. Identification of tannin compounds

The thick extract was weighed 0.1 g and mixed with 10 ml of distilled water. Then shaken and filtered. The filtrate obtained was dripped with three drops of FeCl solution. Positive extracts contain tannin by changing the color of the solution to blackish blue or blackish green (Desinta, 2015).

3.4.5. Media Creation

The growing media to be used are NA (Nutrient Agar) media. Before making media, first calculate the overall need for NA media to use. After calculating the media requirements to be used, then calculate NA media powder and aquadest to be used.

$$Total\ media\ used\ (ml) \times \frac{1\ g}{50\ ml}$$

After counting, the amount of NA powder needed is 3.9 g and dissolved with distilled water as much as 195 ml in Erlenmeyer. Then the tube covered with aluminum foil and pressed with rubber. Finally cooked while sterilized in an autoclave with a temperature of 121^{o} C for 15 minutes and the media is ready to be poured into a test tube and petri dish.

3.4.6. Rejuvenation Bacteria

Colonies of *Staphylococcus aureus, Staphylococcus epidermidis,* and *Propionibacterium acnes* bacteria are obtained from the Laboratory of the Microbiology University of Darussalam Gontor. Next, bacterial rejuvenation is carried out by pouring 5 ml NA media into the test tube then they are tilted and left to solidify in the refrigerator. Then two needles of the *Staphylococcus aureus, Staphylococcus epidermidis*, and *Propionibacterium acnes* were added in each test tube. The test tube was covered with cotton and warp plastic and put into an incubator at 37° C for 24 hours for *Staphylococcus aureus* and *Staphylococcus*

epidermidis, and *Propionibacterium acnes* for 48 hours (Borman *et al*., 2015).

3.4.7. Preparation of Bacterial Suspension

The bacterial culture that has been rejuvenated are made a suspension of bacteria. Each is taken one colony using sterile round ose then suspended in 5 mL of 0.9% NaCl with a vortex. Then the ment of measure the turbidity of bacterial suspensions made at a wavelength of 580 nm to the transmittance of 25% was obtained following Mc Farland solution 1 (Ditjen POM, 1995).

3.4.8. Testing the Antibacterial Activity against Bacterial Spray Gel Preparations

Microbiological test to determine the antibacterial activity of ethanol extract of cherry leaves is performed by the agar diffusion method using the wells by measuring the diameter of bacterial growth inhibition against *Staphylococcus aureus, Staphylococcus epidermidis,* and *Propionibacterium acnes*.

Poured the NA 20 mL media into nine Petri dishes and stored them in the refrigerator for 24 hours to solidify. After the media were uncontaminated, the bacterial suspension was 0.1 mL and flattened using a glass spreader. Then made a pit of 5 holes with a diameter of 5 mm each using a cork borer and then the ingredients to be tested were 50 µL. Each hole is inserted with spray gel with an ethanolic extract from cherry leaves, positive control in the form of clindamycin gel 1% and negative control in the form of spray gel without extract. Clindamycin 1% antibiotic is used as a positive control because clindamycin is the most effective in the treatment of zits when compared to erythromycin and tetracycline and is the most widely used for treating acne (Aziz, 2010). In addition, negative control uses a gel without extract to prove that the base gel has no antibacterial activity at all. They are then incubated for 24 hours at 37^{o} C. The antibacterial activity tests refer to

Borman et al. (2015) with modifications.

Furthermore, the observed area clear zone around the wells and measured using calipers. A broad zone of inhibition was calculated using the following formula (Warbung et al., 2014):

$$Diameter\ of\ the\ inhibition\ zone = \frac{d1 + d2}{2} - x$$

Information:
d1: vertical diameter clear zone on the media (mm)
d2: horizontal diameter clear zone on the media (mm)
x: diameter wells (mm)

The criteria of antibacterial strengths are as follows: inhibition zone with diameter of 5 mm or less then is categorized weak inhibitory activity, inhibition zone with diameter of 5-10 mm can be categorized into moderate, inhibition zone with diameter of 10-20 mm can be categorized strong and if a diameter of 20 mm or greater, it can be categorized as very strong inhibitory activity (Davis & Stout, 1971).

Table 3. Inhibition zone category size

The diameter of the inhibition zone	Inhibition of growth
> 20 mm	Very strong
10-20 mm	Strong
5-10 mm	Moderate
<5 mm	Weak

3.5.Data Analysis

Data from the research results of spray gel preparations of cherry leaves ethanol extract on *Staphylococcus aureus, Staphylococcus epidermidis*, and *Propionibacterium acnes* were analyzed computer software and tested for normality. If data is not normally distributed, then the Kruskal-Wallis method is used and tested further with Man-Whitney. This data analysis uses a significance value of 95% ($\alpha = 0.05$). The Kruskal-Wallis method aims to determine the meaningful differences in the overall samples in inhibiting the growth of *Staphylococcus aureus, Staphylococcus*

epidermidis and *Propionibacterium acnes*. Whereas the aim of the post-hoc test or Mann-Whitney aims to determine the difference in the effectiveness of the gel spray preparation against the bacteria.

CHAPTER IV

RESULTS AND DISCUSSION

4.1.Phytochemical Screening

The thick extract from maceration has the physical characteristic such as its color is deep brown, the smell is distinctive, the shape is very thick and sticky, and it tastes bitter. The result of calculating the yield was 19.6% with the result of water content were 0.0783%. The extract obtained was tested for phytochemical content to determine the chemical content contained in the extract. Phytochemical screening results of cherry leaves ethanol extract are:

Table 4. Phytochemical screening results

Phytochemical content	Result	Conclusions
Flavonoid	Orange formed	+
Alkaloid	Brown formed	+
Saponin	Foam appears	+
Tannin	The solution changes color to blackish green	+

Information: + = Shows positive results

Qualitative testing of the presence of these metabolites means that 70% ethanol extract of cherry leaves positively contains flavonoids, alkaloids, saponins, and tannins. These results are in line with the previous study from Buhian (2016) which stated that the ethanol extract of cherry leaves contained phytochemical compounds in the form of sterols, flavonoids, alkaloids, saponins, glycosides, and tannins. The positive result of flavonoids is the appearance of yellow or orange when the extract added with NaOH. This happens because the chrysine compound which is a derivative of the flavone compound reacts with NaOH to break down due to NaOH alkalinity. The result is that the flavone compounds break down into several molecules, one of which is the yellow color acetophenone (Achmad, 1986).

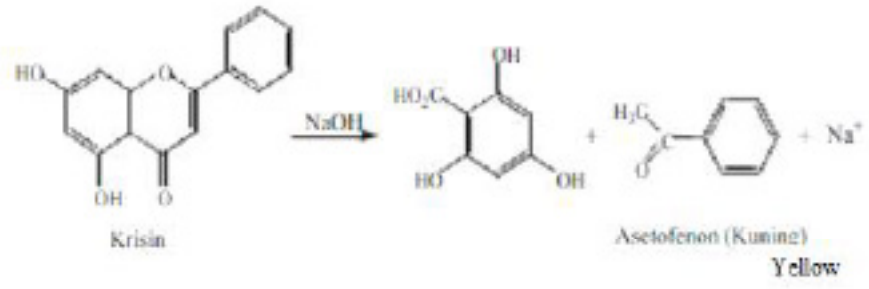

Figure 11. Flavonoid test reaction (Achmad, 1986).

The sign of the presence of alkaloid compounds in the extract is the formation of brown or red deposits when the extract is added with the Wagner reagent (Kl + I_2). The appearance of these deposits occurs because the ligand is in the form of a transition group metal in a reagent with an alkaloid compound. If a reaction occurs, brown or red deposits appear which prove the presence of alkaloids in the extract (Nafisah et al., 2014).

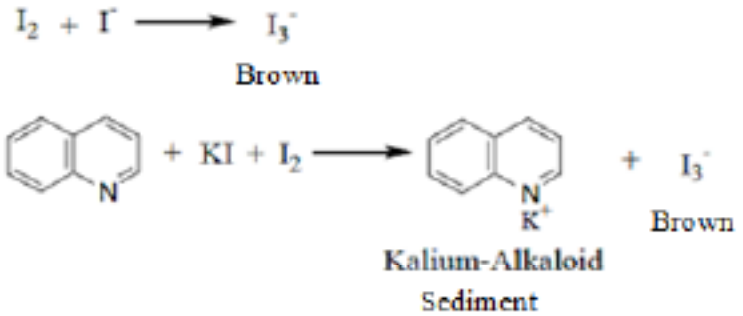

Figure 12. Alkaloid test reaction (Nafisah et al., 2014).

The presence of saponin in the extract is evidenced by the foam or foam that appears after the shaking done. This reaction happens because of the hydrolysis reaction of the saponin compound so that the structure of the aglycone and the glicon is broken to cause foam or foam (Marliana et al., 2005).

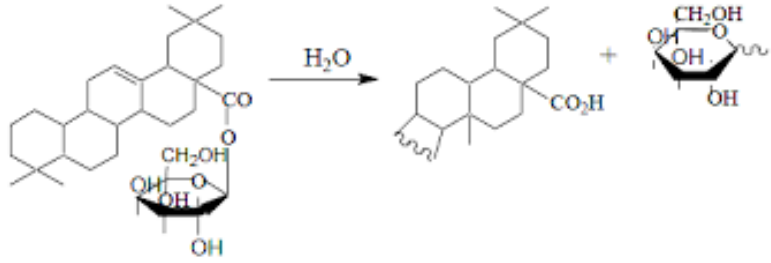

Figure 13. Saponin test reaction (Marliana et al., 2005).

The positive results of the presence of tannin in the extract can be known by the color change to blackish blue or blackish green when added FeCl. This reaction happens because of the reaction between the phenol group contained in the chemical structure of tannins and FeCl. If it reacts to each other, the complex will form a blue or blackish green (Desinta, 2015).

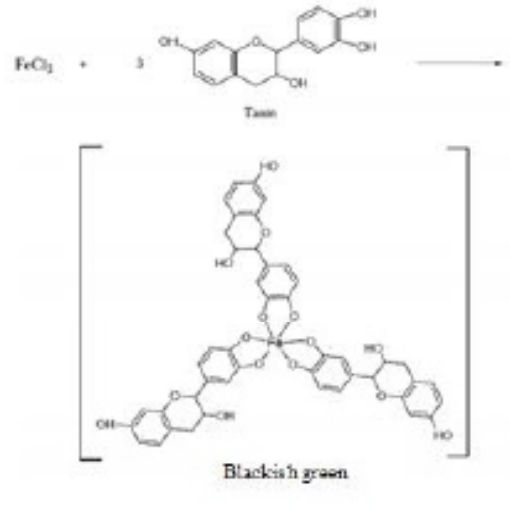

Figure 14. Tannin test reaction (Marliana et al., 2005)

4.2.Antibacterial Activity Test

The spray gel that has been formulated then tested its antibacterial activity against three bacteria that cause acne namely *Staphylococcus aureus, Staphylococcus epidermidis,* and *Propionibacterium acnes*. The

test method used is the agar diffusion method using wells. The working principle of the method of good diffusion is to give time to the test sample to diffuse into the surrounding namely to the test bacteria. Furthermore, it has seen the growth of test bacteria in the diffused area of the test sample.

After a clear zone is formed around the well, measurements are made using a ruler or caliper. The data are processed so that it can be seen whether there is a different activity from formula to each bacterium. The data obtained were tested for normality with normality tests. After being tested, the results showed that the data obtained had a value of $p < 0.05$, so that the data could not be normally distributed (Appendix 6). Since it not normally distributed, the data cannot be processed using the ANOVA test, therefore an alternative test is selected namely the Kruskal-Wallis test. The ANOVA test and the Kruskal-Wallis test the same purpose, namely to find out the difference between 2 or more variables. The difference is, ANOVA is used in data that are normally distributed, while Kruskal-Wallis is for data that is not normally distributed (Dahlan, 2017).

The results of the Kruskal-Wallis test showed that the test results of the test samples on *S.aureus, S.epidermidis*, and *P.acnes* bacteria had p values <0,05 (Appendix 7). It means that the whole test sample has a significant difference in inhibiting the growth of bacteria that cause acne. The Mann-Whitney follow-up test was then carried out to determine the differences in the antibacterial activity of each test sample against each test bacterium (Appendix 8).

Table 5. Results of Mann-Whitney Analysis

Test bacteria	Sample test	Mean of the diameter of inhibition zone ± SD	Inhibition category
S.aureus	F	$8,83 \pm 0,44^{a}$	Medium
	K-	0 ± 0^{b}	Nothing
	K+	$26,67 \pm 0,58^{c}$	Very strong
S.epidermidis	F	$8,28 \pm 0,38^{a}$	Medium
	K-	0 ± 0^{b}	Nothing
	K+	$25,33 \pm 0,29^{c}$	Very strong

P.acnes	F	$8,22 \pm 0,63^{a}$	Medium
	K-	0 ± 0^{b}	Nothing
	K+	$26,33 \pm 0,29^{c}$	Very strong

Description: numbers followed by superscripts with different letters (a,b,c) mean are significantly different ($p < 0,05$) based on testing using SPSS 17 with the Mann-Whitney test method and a 95% confidence level ($\alpha = 0,05$).

Information:

F: Formula spray gel with extract

K+: Positive control

K-: Negative control

The table above shows that spray gel formula and positive controls in the form of clindamycin 1% have antibacterial activity with evidence of a clear zone around the well. The clear zone indicates that the test sample has a substance that can inhibit or kill the bacteria tested. In this study, the spray gel formula is categorized as having moderate strength antibacterial activity because of the diameter of the inhibition zone in the range of 5-10 mm (Davis & Stout, 1971). Meanwhile clindamycin which has an average diameter of the inhibitory zone is categorized very strongly because the inhibition zone is > 20 mm. In addition, the negative control in the form of a spray gel base without extract showed no inhibition zone formed, meaning the gel base used in the formulation had no antibacterial activity.

When spray gel formula is compared with positive controls, there are significant differences in antibacterial activity. Spray gel formula has moderate category of antibacterial activity and clindamycin is in the very strong category. This activity means that the antibacterial activity of spray gel formula cannot be compared with clindamycin as a comparison. This also happened in the Wulandari (2017) study, which was caused by clindamycin as a pure compound, while the spray gel of the ethanol extract from cherry leaf was still an extract whose content was still various. Compounds other than flavonoids, alkaloids, saponins, and tannins can interfere with the potential antibacterial activity in them.

From the results of the observation, it can be seen that the spray gel formulas did not become significant in their antibacterial activity in inhibiting *S. aureus, S.epidermidis, and P.acnes* bacteria. This equation is due to the similarity in the three bacteria test. The three bacteria are the Gram + group of bacteria that have a thick and relatively simple wall arrangement compared to the bacterial Gram – group wall which is thin but complicated. The cell wall of Gram + consists of peptidoglycan components, small lipid, and polysaccharides (taikonaut acid). Taikonaut acid is a water-soluble polymer that functions as a positive ion transport to enter and exit bacterial cells. Because of this water solubility, it can be seen that the cell walls of gram + bacteria are more polar. The bacteria cell wall is polar so that they be easily damaged by extracts which are also polar (Fissy et al., 2014).

The same as the positive control which gave rise to the insignificant inhibition zone diameter (no difference) in each bacterial test. The reason is the same as the spray gel formula, which is the same as the test bacteria group, namely Gram + bacteria. Besides the same wall shape, the log phase of *S.aureus, S.epidermidis,* and *P.acnes* bacteria has similarities. The phase log is the phase of continuing rapid cell division at a constant rate (Jauhari, 2010). This Phase log is a phase that is suitable for antibacterial testing because the bacterial cell wall is thinned since it actively carries out cell division.

In a research by Saraswati (2015), the log phase of *S. aureus* occurred at the 3rd to the 15th hour. The log phase of *S.epidermidis* and *P.acnes* is at the 4th to 9th hour. The similarity in the three test bacteria is that the log phase is less than 24 hours. The phase after the log phase is the stationary phase and the decreases phase in which it is estimated that at 24 hours when the treatment is carried out, the three bacteria will reach the decrease phase by stopping reproducing themselves and increasing mortality. Because all three experience a decrease phase, the result of the diameter of the inhibition zone will be the same.

The overall antibacterial activity of the spray gel is thought to originate from the content of secondary metabolites which are present in the ethanol extract of cherry leaves. After the phytochemical content was tested, the extract positively contained flavonoids, alkaloids, saponins, and tannins. How it works as flavonoids is by inhibiting nucleic acid synthesis, disrupting the function of the cytoplasmic membrane, and inhibiting energy metabolism from bacteria (Cushnie & Lamb, 2005). The mechanism of flavonoids in inhibiting nucleic acid synthesis using ring B is on flavonoids forming hydrogen bonds with an array of nucleic acid base acids. Since nucleic acid is a constituent of DNA and RNA, the result is that its formation is also disrupted. Thus the nucleus of the bacterial cell becomes damaged and dies (Mori et al., 1987)

The mechanism of flavonoids in disrupting the function of the cytoplasmic membrane is by reducing the fluidity of bacterial cell membranes. The hydrophilic and hydrophobic nature of the cell membrane are disrupted so that it is no longer able to maintain the cell nucleus from foreign substances outside the cell and to keep the inside from coming out. As a result, the outermost bull cells are damaged, and the cell nucleus exits and causes cell death (Tsuchiya & Iinuma, 2000). Next is the way flavonoids inhibit energy metabolism by inhibiting the use of oxygen by bacteria. This oxygen functions as energy which will be metabolized first. It happens because the oxygen to be metabolized is inhibited, energy is not formed, and the biosynthesis of macromolecules in bacteria is disrupted (Cushnie & Lamb, 2005).

The mechanism of action of alkaloids as antibacterial is by disrupting peptidoglycan which is a constituent of bacterial cell walls. Base groups containing nitrogen in alkaloids can damage the structure of amino acid compounds in the cell wall. As a result, the DNA chain in the cell wall is damaged and causes lysis of the cell wall to cause cell death (Juliantina et al., 2008). The mechanism of action of saponin as antibacterial is to leak the contents in the cell until it comes out and dies. Saponins that have

properties such as detergents stick to the surface of the cell wall of bacteria that are lipophilic. After binding, the permeability of the cell wall becomes damaged and results in an uncontrolled outflow of foreign substances from the outside. Because the cell wall is damaged, the contents of bacterial cells come out and cause cell death (Utami, 2008).

Furthermore tannins works as an antibacterial by inactivating enzymes in bacteria. Protein which is a constituent of enzymes is disturbed by tannins by forming complex compounds until hydrogen bonds occur. This hydrogen bond causes the protein to denature and results in the disruption of all activities involving the enzyme in the process. Finally, cell metabolism is disrupted and causes a decrease in bacterial growth (Rijayanti, 2014).

4.3.Halal Product Analysis

The standard of halal products according to the Indonesian Ulema Council (MUI) is the longest and most protected material, process, tool and location of something that is prohibited by Islamic law. Thus, the researcher conducted a product analysis that was formulated to ensure that the products made are safe and *syar'i* to be used by all people especially Muslims. The following is a presentation of the halal analysis of products formulated in Table 6.

Table 6. Halal product analysis

Halal identification		**Identification**		**Information**
		Non-halal	**Halal**	
Ingredients	Fresh cherry leaves		√	
	Cherry leaf extract		√	
	Ethanol 70%		√	Used as a solvent in extraction, but all evaporated and concentrated until it runs out.
	Test bacteria		√	

Process	Extraction		√	
	Spray gel formulation		√	
Tools	Glass and iron laboratory equipment		√	
	LAF		√	
	Incubator		√	
Location	Laboratory		√	

From the halal analysis, it can be seen that almost all materials, processes, tools, and locations are free from something that is forbidden in Islamic law except in the use of alcohol in the extraction process. The opinions of scholars on alcohol in pharmacy are different. Some say it is allowed because alcohol and *khamr* are different on both the structure and the intoxicating effect. Others say it is prohibited based on the words of the Prophet Muhammad, "Every intoxicating thing is *khamr*, and every intoxicating thing is *haram*, something intoxicating, even if it is only little is haram". HR. Ahmad ibn Hanbal, Ibn Majah, and al Daruqutni. Therefore, if there is a lot of alcohol in the product, in it is still haram in its use. To avoid the worst possibility of the law of using formulated products, the researcher prefers to eliminate the entire alcohol used.

Meanwhile, alcohol used is 70% alcohol as a solvent in the extraction process, not as the main ingredient. The last step in the extraction process is evaporation of solvents using the evaporator. In this process, the entire solvent used will evaporate until it is lost, including water. The next step is concentrating the extract with a waterbath to remove the remaining solvents extracted. This aims to remove water levels or other solvents so that the extract will not be easily damaged due to excessive water content. Apart from these reasons, this process also ensures that the alcohol solvent used is completely gone.

In addition to alcohol, the entire materials, processes, tools, and locations used in testing are following Islamic law because they are free from unclean materials and harmful substances such as microorganisms. Thus, the formulated products can be considered *halal toyyiban* products because they are according to Islamic law and have benefits for users.

CHAPTER V

CONCLUSION AND SUGGESTION

5.1. Conclusions

From the formulation of the problem in this study, it can be concluded as follows:

1. The spray gel preparation of the ethanol extract from the cherry leaves can inhibit the growth of *Staphylococcus aureus, Staphylococcus epidermidis,* and *Propionibacterium acnes.*
2. The three spray gel preparations of ethanol extract of cherry leaves have antibacterial activity against the *Staphylococcus aureus, Staphylococcus epidermidis* and *Propionibacterium acnes* bacteria in the moderate category.
3. There is no difference in the level of inhibition from spray gel preparations of ethanol extract of cherry leaves against *Staphylococcus aureus, Staphylococcus epidermidis* and *Propionibacterium acnes.*

5.2. Suggestions

For the results of this study to be more accomplished, it is necessary to conduct further research including:

1. Using specific secondary metabolite isolates from cherry leaves extract and eliminating other components that have less effect on antibacterial activity. Hence, the activity is expected to be better and to offer antibiotics from chemicals.
2. It needs further testing of antibacterial activity of spray gel with an ethanolic extract from cherry leaves against Gram-negative bacteria

BIBLIOGRAPHY

Achmad, S. (1986). *Kimia Organik Bahan Alam.* Jakarta: Karunia Jakarta.

Agoes, G. (2012). *Sediaan Farmasi Likuida-Semisolida (SFI-7).* Bandung: Penerbit ITB.

Agoes, G. (2015). *Sediaan Kosmetik (Seri Farmasi Industri-9).* Bandung: Penerbit ITB.

Ajizah, A. (2004). *Sensitivitas Salmonella Typhimurium Terhadap Ekstrak Daun Psidium Guajava L.* . Kalimantan: Universitas Lambung Mangkurat Press.

Alkhakim, F., Huda, M., Fitri, G., Ambarwati, D., & Tistiana, H. (2013). Pengaruh Ekstrak Daun Kersen Terhadap Daya Tetas dan Mortalitas Telur Itik Hibrida.

Apriliyanti, E. (2016). *Uji Aktivitas Antibakteri Ekstrak Etanolik Daun Kersen (Muntingia calabura L.) Terhadap Propionibacteriu acnes Secara In Vitro (Skripsi).* Semarang: Unissula.

Arum, Y., Supartono, & Sudarmin. (2012). Isolasi dan Uji Daya Antimikroba Ekstrak Daun Kersen. *Jurnal MIPA*, Vol 35(2): 167-174.

Ayu, S. (2009). *Cara Ampuh Mengobati Jerawat.* Yogyakarta: Buana Pustaka.

Aziz, S. (2010). *Uji Aktivitas Antibakteri Ekstrak Etanol Daun dan Umbi Bakung Putih (Crinum asiatikum L.) Terhadap Bakteri Penyebab Jerawat (Skripsi).* Jakarta: Universitas Islam Negeri Syarif Hidayatulloh.

Borman, I., Yusriadi, & Sulastri, E. (2015). Gel Antijerawat Ekstrak Daun Buta-Buta (Excoecaria agollocha L.) dan Pengujian Antibakteri Staphylococcus epidermis. *Gelenika Journal of Pharmacy*, Vol. 1(2): 65-72.

Brook, G., Butel , J., & Morse, S. (2005). *Mikrobiologi Kedokteran.* Jakarta: Salemba Medika.

Buhian, W., Rubio, R., Valle, D., & Puzon, J. (2016). Bioactive Metabolite Profiles and Antimicrobial Activity of Ethanolic Extract of Muntingi calabura L. Leaves and Stems. *Asian Pacific Journal of Tropical Biomedicine*, Vol 6(8): 682-685.

Burcio, O. (2017, Oktober). *Saponin-Amphipathic Glycosides*. Retrieved from NaToxAq: https://natoxaq.ku.dk

Cunliff, W. e. (2001). *Acne Diagnosis and Management.* London: Martin Dunitz Ltd.

Cushnie, T., & Lamb, A. (2005). Review: Antimicrobial Activity of Flavonoids. *International Journal of Antimicrobial Agents 26*, 343-356.

Dahlan, M. (2017). *Statistik untuk Kedokteran dan Kesehatan: Deskriptif, Bivariat, dan Multivariat.* Jakarta: Epidemiologi Indonesia.

Davis, W., & Stout, T. (1971). *Disc Plate Methods of Microbiology.*

Depag. (2003). *Petunjuk Teknis Pedoman System Produksi Halal.* Jakarta: Departemen Agama.

Depkes RI. (1986). *Sediaan Galenik.* Jakarta: Departemen Kesehatan RI.

Desinta, T. (2015). Penentuan Jenis Tanin Secara Kualitatif dan Penetapan Kadar Tanin dari Kulit Buah Rambutan (Naphelium lappaceum L.) Secara Permanganometri. *Ujrnal Ilmiah Mahasiswa Universitas Surabaya, Vol.4 No.1*, 1-10.

Devi, N., Miranti, I., & Wijayahadi, N. (2016). Pengaruh Ekstrak daun Kersen (Muntingia calabura) Terhadap Gambaran Mikroskopis Ginjal Tikus Wistar Jantang yang Diinduksi Etanol dan Soft Drink. *Jurnal Kedokteran Diponegoro*, Vol.5(4): 658-664.

DitjenPEN, D. J. (2014). Obat Herbal Tradisional. *Warta Ekspor*.

DitjenPOM. (1995). *Farmakope Indonesia Edisi IV.* Jakarta: Depkes RI.

DitjenPOM. (2000). *Parameter Standar Umum Ekstrak Tumbuhan Obat.* Jakarta: Depkes RI.

DitjenPOM. (2014). *Farmakope Indonesia, Edisi V.* Jakarta: Kemenkes RI.

Dwidjoseputro. (1998). *Dasar-Dasar Mikrobologi.* Jakarta: Djambatan.

Encyclopaedia Britannica. (2018, Agustus). *Alkaloids*. Retrieved from Encyclopaedia Britannica: www.britannica.com

Fissy, S., Sari, R., & Pratiwi, L. (2014). Efektivitas Gel aantijerawat Ekstrak Etanol Rimpang Jahe Merah (Zingiber officinale Rosc. Var. Rubrum) Terhadap Propionibacterium Acnes dan Staphylococcus epidermis. *Farmakon Jurnal Ilmu Kefarmasian Indonesia*, Vol. 12(2): 193-201.

Fitriansyah, S. (2016). Formulasi Dan Evaluasi Spray Gel Fraksi Etil Asetat Pucuk Daun Teh Hijau (Camelia sinensis [l.] Kuntze) Sebagai Antijerawat. Pharmacy, Vol.13 No. 02. *Pharmacy Journal*, Vol.13, No.02.

Guenther, E. (2006). *Minyak Atsiri, Jilid I.* Jakarta: UI-Press.

Hanani, E. (2015). *Analisis Fitokimia.* Jakarta: Penerbit Buku Kedokteran EGC.

Handayani, F., & Sentat, T. (2016). Uji Aktivitas Ekstrak Etanol Daun Kersen (Muntingia calabura L.) Terhadap Penyembuhan Luka Bakar pada Kulit Mencit Putih Jantan (Mus musculus). *Jurnal Ilmiah Ibnu Sina* , Vol 1(2): 131-142.

Handayani, V. (2014). Pengujian Aktivitas Antibakteri Ekstrak Etanol Daun Kersen (Muntingia calabura L.) Terhadap Bakteri Penyebab Jerawat. *Jurnal Fitofarmaka Indonesia,*, Vol 2(1): 94-96.

Harborne, J. (1987). *Metode Fitokimia, Penuntun Cara Modern menganalisis Tumbuhan.* Bandung: Penerbit ITB.

Hart, T., & Shears, P. (2004). *Atlas Berwarna Mikrobiologi Kedokteran.* Jakarta : Hipokrates.

Harti, A. (2015). *Mikrobiologi Kesehatan.* Yogyakarta: Penerbit Andi.

Heinrich, M., Barner, J., Gibbons, S., & Williamson, E. (2009). *Farmakognosi dan Fitoterapi.* Jakarta: Penerbit Buku Kedokteran EGC.

Hidayat, S., & Napitupulu, R. (2015). *kitab Tumbuhan Obat.* Jakarta: AgriFlo.

Hutapea, J. (1994). *Inventaris Tanaman Obat Indonesia (III).* Jakarta: DepKes RI.

Ikhsanudin, A., & Mardhiyah, S. (2017). Formulasi dan Uji Antijerawat Gel Ekstrak Etanol 70% Buah Belimbing Wuluh (Averrhoa bilimbi Linn.) Terhadap Bakteri Propionibacterium acnes . *Fakultas Farmasi Universitas Ahmad Dahlan*, Vol. 5(1): 416-426.

Irianto, K. (2013). *Mikrobiologi Medis* . Bandung: Penerbit Alfabeta.

Jauhari, L. (2010). *Seleksi dan Identifikasi kapang Endofit Penghasil Antimikroba Penghambat Pertumbuhan Mikroba Patogen (Skripsi)* . Jakarta: Universitas Islam Negeri Syarif HIdayatullah.

Jauregui, K., Gragorio, Cabrera, J., Ceniceron, E., Hernandez, J., & Ilyina, A. (2009). A New Formulated Stable Papin-Pectin Aerosol Spray for Skon Woundhealing. *Biotechnology and Bioprocess Enginering*, Vol. 14: 450-456.

Jewetz, M., & Adelberg. (2012). *Mikrobiologi Kedokteran Edisi 25.* Jakarta: Penerbit Buku Kedokteran EGC.

Jewetz, M., & Adelberg's. (2010). *Mikrobiologi Kedokteran* . Jakarta: Buku Kedokteran EGC.

Juliantina, F., Citra, D., & Nirwani, B. (2008). *Manfaat Sirih Merah (Piper crocatum) Sebagai Agen Anti Bakterial Terhadap Bakteri Gram Positif dan Gram Negatif.* Yogyakarta: UII Press.

Kligman, L. (1994). Identification of Salmonella typhii. *Journal of Microbiology*, 288-300.

Kurokawa, I., Danby, F., Ju, Q., & Wang, X. (2009). New Developments in Our Understanding of Acne Pathogenesis and Treatment. *Experimental Dermatology*, Vol 18.

Kusnaeni, V. (2008). *Isolasi dan Karakterisasi Senyawa Fraksi n-Heksana dari Ekstrak Kulit Batang Angsret (Spathoda campanulata Beauv)*

(Skripsi). Malang: Universitas Brawijaya.

Kwangshin, K. (2015). *Propionibacterium acnes*. Retrieved from ScienceSourceImages: https://www.sciencesource.com

Lachman, L., Herbert, A., Lieberman, Joseph, L., & Kanig. (1994). *Teori dan Praktek Farmasi Industri.* Jakarta: UI Press.

Laianto, S. (2014). Uji Efektivitas Sediaan Gel Anti Jerawat Ekstrak Etanol Buah Pare (Momordica Charantia) Terhadap Staphylococcus epidermis dan Propionibacterium acnes dengan Metode Difusi (Skripsi). Pontianak: Universitas Tanjungpura.

Lambert, B. (2013, Januari). *Condensed Tannins in the Ruminant Environment: A Perspective on Biological Activity*. Retrieved from ReserchGate: https://www.researchgate.net

Lutz, D. (2015, Januari). *Staphylococcus aureus*. Retrieved from ScienceDaily: https://www.sciencedaily.com

Marliana, S., Suryanti, & Suyono. (2005). *Skrining Fitokimia dan Analisis Kromatogafi Lapis Tipis Komponen Kimia Buah Labu Siam (Sechium edule Jacq. Swartz.) dalam Ekstrak Etanol.* Surakarta: Universitas Sebelas Maret.

Marliana, S., Suryanti, & Suyono. (2005). *Skrining Fitokimia dan Analisis Kromatogafi Lapis Tipis Komponen Kimia Buah Labu Siam (Sechium edule Jacq. Swartz.) dalam Ekstrak Etanol .* Surakarta: Universitas Sebelas Maret.

Miksusanti, Betty, S., Rizal, S., Bambang, P., & Gatot, T. (2009). Antibacterial Activity of Temu Kunci Tuber (Kampheria pandurata) Essential Oil Againts Bacillus cereus. *Med J Indones*, Vol 18, No 1:11.

Monica, W., Mahatmi, H., & Besung, K. (2013). Pola Resistensi Salmonella typhi yang Diisolasi dari Ikan Serigala (Hoplias malabaricus) terhadap Antibiotik. *Jurnal Ilmu dan Kesehatan Hewan*, 64-69.

Mori, A., Nishino, C., Enoki, N., & Tawata , S. (1987). Antibacterial Activity adan Mode of Action of Plant Flavonoids Againts Proteus vulgaris

and Staphylococcus aureus . *Phytochemistry*, 2231-2234.

Movita, T. (2013). Acne Vulgaris . *Continuing Medical Education*, Vol 40(3): 269-272.

Muhlisah, F. (2000). *Tanaman Obat Keluarga.* Jakarta: Penebar Swadaya.

Nafisah, M., Tukiran, Suyatno, & Hidayati, N. (2014). *Uji Skrining Fitokimia pada Ekstrak Heksan, Kloroform dan Metanol dari Tanaman Patikan Kebo (Euphorbiae hirtae).* Surabaya: Prosiding Seminar Nasional Kimia Universitas Negeri Surabaya.

NIAID. (2011, April). *Staphylococcus epidermidis*. Retrieved from https://www.flickr.com

Nisak, K. (2016). *Uji Stabilitas Fisik dan Kimia Sediaan Gel Semprot Ekstrak Etanol Tumbuhan Paku (Nephrolepis falcata (Cav.) C. Chr.).* Jakarta: UIN Syarif Hidayatullah Jakarta.

Nurhasanah, N. (2012). *Isolasi Senyawa Antioksidan Ekstrak Metanol Daun Kersen (Muntingia calabura L.).* Cimahi: Universitas Jenderal Achmad Yani.

Oprica, C. (2004). Antibiotic Resistant Propionibacteriumacnesnon the Skin of Patient with Moderate to Severe Acne . *Journal of Pharmacology*, Vol 10(3): 155-164.

Pakaya, W., Ischak, N., & Tangio, j. (2015). *Analisis Kadar Flavonoid dari Ekstrak Metanol Daun dan Bunga Temebelekan.* Gorontalo: Universitas Negeri Gorontalo.

Pakaya, W., Ischak, N., & Tangio, J. (2015). *Analisis Kadar Flavonoid dari Ekstrak Metanol Daun dan Bunga Temebelekan.* Gorontalo: Universitas Negeri Gorontalo.

Pandey, A. (2013, Desember). *Chemistry and Biological Activities of FlavonoidsL An Overview*. Retrieved from ResearchGate: https://www.researchgate.net

Price, S., & Lorraine, M. (2006). *Patofiologi Konsep Klinis Proses-Proses Penyakit, Edisi 6.* Jakarta: Penerbit Buku Kedokteran EGC.

Purwonegoro, I. (1997). Uji Sitotoksisitas dari Ekstrak Heksan, Etil Asetat dan Etanol 70% dari Akar Daun Kersen (Muntingia calabura L.) Terhadap Artemia Salina (LEACH) dan Skrining Kandungan Kimianya (Skripsi).

Putri, C., Yuliet, & Khaerati, K. (2018). Efektivitas Ekstrak Daun Kersen (Muntingia calabura L.) Terhadap Penurunan Kadar Kolesterol Total Tikus Putih Jantan (Rattus norvegicus L.) yang Diinduksi Pakan Tinggi Lemak. *Biocalabes*, Vol 12(1): 65-72.

Putri, D. (2016). Pengaruh pemberian Ekstrak Daun Kersen (Muntingia calabura) Terhadap Lalat Buah Bactrocera carambolae. *Al-Kauniyah: Journal of Biology*, Vol 9(2): 139-143.

Qardhawi, Y. (2007). *Halal dan Haram dalam Islam.* Surakarta: Era Intermedia.

Radji, M. (2011). *Buku Ajar Mikrobiologi Panduan Mahasiswa Farmasi dan Kedokteran.* Jakarta: Penerbit Buku Kedokteran EGC.

Ramasamy, R., Nanjundan, J., Smitha, K., & Ponnusamy, M. (2017). Screening of Antibacterial Activity of Muntingia calabura Leaves Extract Againts Bacterial Pathogens. *International Journal og Chemical Studies*, 313-316.

Ratnasari, M. (2017). *Uji Aktivitas Antibakteri Ekstrak Daun Kersen (Muntingia calabura L.) Dalam Bentuk Sediaan Gel Terhadap Staphylococcus aureus dan Escheriachia coli (Jurnal Skripsi).* Yogyakarta: Universitas Atma Jaya Yogyakarta.

Rijayanti, R. (2014). *Uji Aktivitas Antibakteri Ekstrak Etanol Daun Mangga Bacang (Mangifera foetida L.) Terhadapa Staphylococcus aureus Secara In Vitro.* Pontianak: Universitas Tanjungpura.

Saifudin, A., Rahayu, V., & Teruna, H. (2011). *Standarisasi Bahan Obat Alam.* Yogyakarta: Graha Ilmu.

Saising, J., Hinanrat, A., Mahabusarakan, W., Ongsakul , M., & Voravuthikunchai, S. (2008). Rhadomyrthone from Rhadomyrtus

tomentosa (Aiton) Hassk. As a Natural Antibiotic for Staphylococcus Cutaneous Infection. *Journal of Health Science*, Vol 54(5): 589-592.

Sami, F., Nur, S., Ramli, N., & Sutrisno, B. (2017). Uji Aktivitas Antioksidan Daun Kersen (Muntingia calabura L.) dengan Metode DPPH (1,1-difenil-e-pikrilhidrazil) dan FRAP (Ferric Reducing Antioxidan Power). *As-Syifaa*, Vol.09(02): 106-111.

Saraswati, F. (2015). *Uji Aktivitas Antibakteri Ekstrak Etanol 96% Limbah Kulit Pisang Kepok Kuning (Musa Balbisiana) Terhadap Bakteri Penyebab Jerawat (Staphylococcus epidermidis, Staphylococcus aureus, dan Propionibacterium acne) (Skripsi).* Jakarta: UIN Syarif Hidayatullah .

Sarlina, Razak, A., & Tandah, M. (2017). Uji Aktivitas Antibakteri Sediaan Gel Ekstrak Daun Sereh (Cymbopogon nardus L. Rendle) Terhadap Bakteri Staphylococcus aureus Penyebab Jerawat. *Jurnal Farmasi Gelanika*, Vol.3(2): 143-149.

Sasanti, T., Wibowo, M., Fidriany, L., & Caroline, S. (2006). formulasi Gel Ekstrak Air Teh Hijau dan Penentuan Aktivitas Antibakterinya Terhadap Propionibacteria acnes (Skripsi). *Sekolah Tinggi ITB*, 8-11.

Seli, M., Wibowo, M., & Arreneuz, S. (2015). Aktivitas Antibakteri Ekstrak Daun Soma (Ploiarium alternifolium Melch) Terhadap Propionibacterium acnes. *JKK*, Vol. 4(4): 72-82.

Sirait, M. (2007). *Penuntun Fitokimia dalam Farmasi.* Bandung: Penerbit ITB.

Steenis, C., Bloembergen, S., & Eyma, P. (2005). *Flora Cetakan ke-10.* Jakarta: PT. pradnya paramita.

Tamu, F. (2017). Formulasi dan Uji efektifitas Antioksidan Krim Ekstrak Etanol Daun Kersen (Muntingia calabura L.) dengan Metode DPPH (Skripsi). *Universitas Islam Negeri Alauddin Makassar*.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur , H. (2011). Phytocemical Screening and Extraction: A Review. *International Pharmaceutica*

Scienca, 96-106.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytocemical Screening and Extraction: A Review. *International Pharmaceutica Scienca*, 96-106.

Tranggono, R., & Latifah, F. (2007). *Buku Pegangan Ilmu Pengetahuan Kosmetik.* Jakarta: Penerbit Pustaka Utama.

Tsuchiya, H., & Iinuma, M. (2000). Reduction of Membrane Fluidity by Antibacterial Sophoraflavone G Isolated from Sophora exigua . *Phytomedicine*, 161-165.

Underwood, J. (2000). *Patologi Umum dan Spesifik, Edisi 2, Volume 2.* Jakarta: Penerbit Buku Kedokteran EGC.

Utami, P. (2008). *Buku Pintar Tanaman Obat* . Tangerang: Agro Media.

Waluyo, L. (2007). *Mikrobiologi Umum.* Malang: UMM.

Warbung, Y., Wowor, V., & Posangi, J. (2014). *Daya Hambat Ekstrak Spons Laut Callyspongia sp. Terhadap Pertumbuhan Bakteri Staphylococcus aureus.* Manado: Unsrat.

Warsa, U. (1994). *Buku Ajar Mikrobiologi Kedokteran.* Jakarta: Penerbit Binarupa Aksara.

Wulandari, S. (2017). Formulasi dan Uji Aktivitas Antibakteri Staphylococcus epidermis Sediaan Mikroemulsi Ekstrak Daun Kersen (Muntingia calabura L.) dengan Fase MInyak Isopropil Mirystate (Skripsi). Malang: Universitas Islam Negeri Maulana Malik Ibrahim .

Yuni, A., Paulina, V., & Hamidah, S. (2013). Formulasi dan Uji Aktifitas Gel Antijerawat Ekstrak Umbi Bakung (Crinum asiaticum L.) Terhadap Bakteri Staphylococcus aureus Secara In Vitro. *Jurnal Ilmiah Farmasi-UNSRAT*, Vol. 2(2): 18-26.

Zakaria, Z., Sufian, A., Ramasamy, K., Ahmat, N., Sulaiman, M., Arifah, A., et al. (2010). In Vitro Antimicrobial Activity of Muntingia calabura Extracts and Fractions. *African Journal of Microbiology Research*, 304-308.

APPENDICES

Appendix 1. Letter of the result of plant determination

UNIVERSITAS GADJAH MADA
FAKULTAS FARMASI
Sekip Utara, Yogyakarta 55281 Telp./Fax. +62 274 543120
http://farmasi.ugm.ac.id, E-mail: farmasi@ugm.ac.id

SURAT KETERANGAN
No.: 8.7.1 /UN1/FFA/BF/PT/2019

Yth. : Aisyah Rulina Safitri 7 Januari 2019
NIM 362015712275
Prodi Farmasi
Fakultas Ilmu Kesehatan
Universitas Darussalam Gontor
Di Ngawi

Bersama ini kami sampaikan hasil identifikasi sampel yang Saudara kirimkan ke Departemen Biologi Farmasi, Fakultas Farmasi UGM, adalah :

No.Pendaftaran	Jenis	Suku
137	*Muntingia calabura* L.	Elaeocarpaceae

Demikian, semoga dapat digunakan sebagaimana mestinya.

Mengetahui,
Dekan

Prof. Dr. Agung Endro Nugroho, M.Si.,Apt

Ketua Departemen Biologi Farmasi

Dr. Indah Purwantini, M.Si., Apt.

Appendix 2. Making simplicia of cherry leaves powder

Figure 1. Fresh cherry leaves

Figure 2. Drying the leaves with an oven

Figure 3. Reducing the size of the leaves with a blender

Figure 4. Uniformity of size with sieve

Appendix 3. Extraction

Figure 1. Transmission using maceration method using 70% ethanol

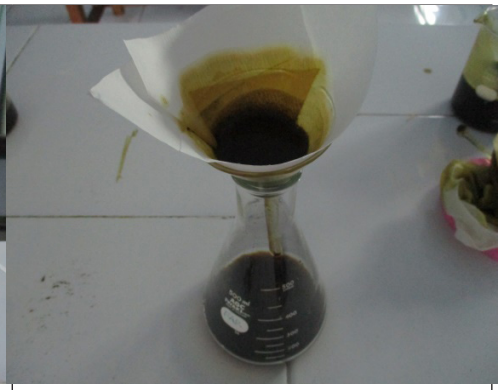

Figure 2. Filtering with filter paper

Figure 3. Maserat 1, 2, and 3

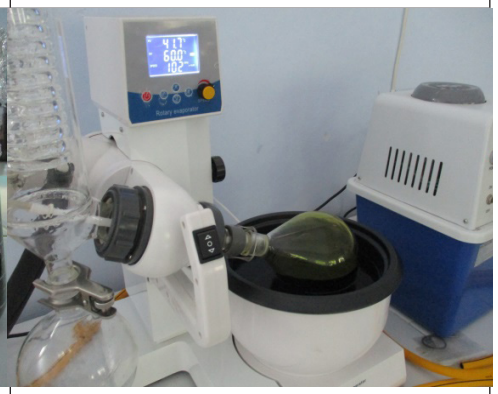

Figure 4. Separation of liquid extract from solvent

Figure 5. Thickening of extract with waterbath

Figure 6. Thick extract

Appendix 4. Identification of phytochemical extract

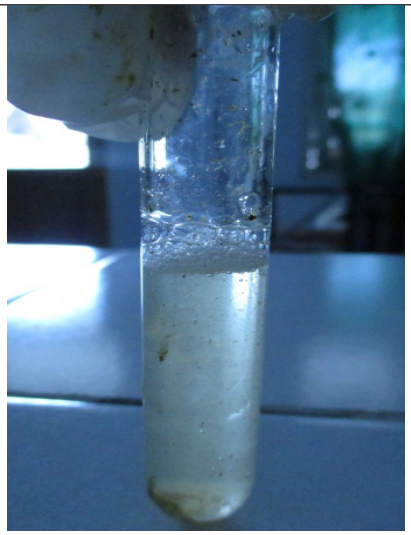

Figure 1. Saponin content test

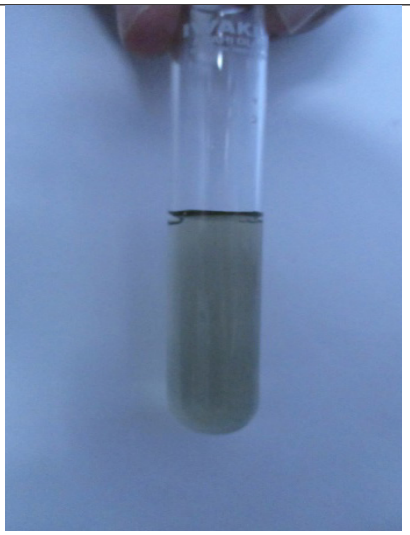

Figure 2. Tannin content test

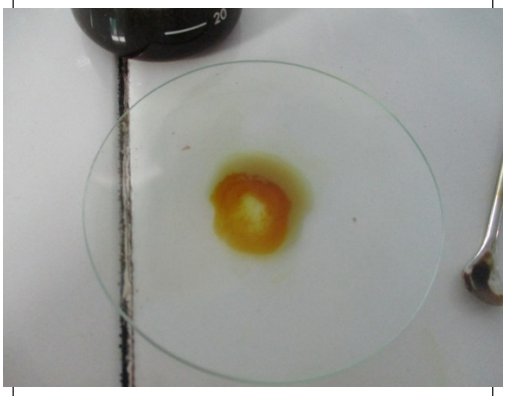

Figure 3. Flavonoid content test

Figure 4. Alkaloid content test

Appendix 5. Antibacterial activity test of spray gel preparations

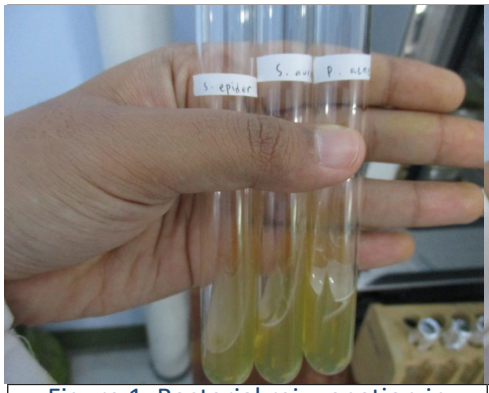

Figure 1. Bacterial rejuvenation in slanted media

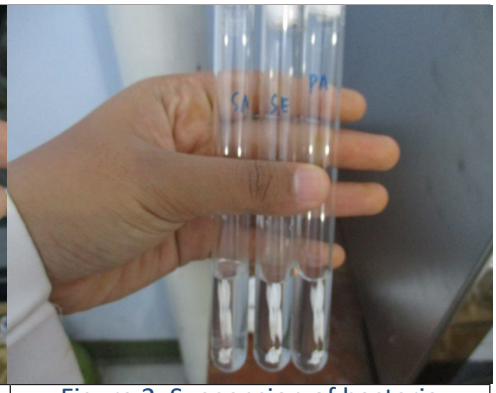

Figure 2. Suspension of bacteria

Figure 3. Antibacterial test in LAF (Laminar Air Flow)

Figure 4. Storage of media test in an incubator

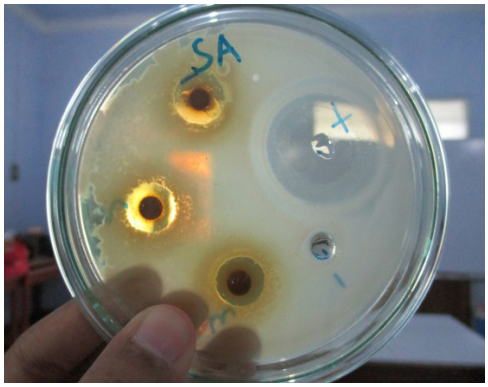

Figure 5. Antibacterial test results

Appendix 6. Normality test

Descriptives[a,b,c]

	Terhadap bakteri		Statistic	Std. Error
Spraygel	S.aureus	Mean	8.3333	.25314
		Std. Deviation	.43844	
	S.epidermidis	Mean	8.2767	.22333
		Std. Deviation	.38682	
	P.acnes	Mean	8.2233	.36498
		Std. Deviation	.63217	
Kontrolpositif	S.aureus	Mean	26.6667	.33333
		Std. Deviation	.57735	
	S.epidermidis	Mean	25.3333	.16667
		Std. Deviation	.28868	
	P.acnes	Mean	26.3333	.16667
		Std. Deviation	.28868	

a. Kontrolnegatif is constant when Terhadap bakteri = S.aureus. It has been omitted.

b. Kontrolnegatif is constant when Terhadap bakteri = S.epidermidis. It has been omitted.

c. Kontrolnegatif is constant when Terhadap bakteri = P.acnes. It has been omitted.

Tests of Normality[b,c,d]

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Terhadap bakteri	Statistic	df	Sig.	Statistic	df	Sig.
Spraygel	S.aureus	.312	3	.	.896	3	.373
	S.epidermidis	.385	3	.	.750	3	.000
	P.acnes	.336	3	.	.856	3	.258
Kontrolpositif	S.aureus	.385	3	.	.750	3	.000
	S.epidermidis	.385	3	.	.750	3	.000
	P.acnes	.385	3	.	.750	3	.000

a. Lilliefors Significance Correction

b. Kontrolnegatif is constant when Terhadap bakteri = S.aureus. It has been omitted.

c. Kontrolnegatif is constant when Terhadap bakteri = S.epidermidis. It has been omitted.

d. Kontrolnegatif is constant when Terhadap bakteri = P.acnes. It has been omitted.

Appendix 7. Kruskal-wallis test

Ranks

	Terhadap bakteri	N	Mean Rank
Spraygel	S.aureus	3	5.33
	S.epidermidis	3	4.67
	P.acnes	3	5.00
	Total	9	
Kontrolpositif	S.aureus	3	7.17
	S.epidermidis	3	2.00
	P.acnes	3	5.83
	Total	9	
Kontrolnegatif	S.aureus	3	5.00
	S.epidermidis	3	5.00
	P.acnes	3	5.00
	Total	9	

Test Statistics[a,b]

	Spraygel	Kontrolpositif	Kontrolnegatif
Chi-Square	.092	5.954	.000
df	2	2	2
Asymp. Sig.	.955	.051	1.000

a. Kruskal Wallis Test

b. Grouping Variable: Terhadap bakteri

Appendix 8. Mann-whitney

Spray gel

Ranks

	Terhadap bakteri	N	Mean Rank	Sum of Ranks
Spraygel	S.aureus	3	3.67	11.00
	S.epidermidis	3	3.33	10.00
	Total	6		

Test Statistics[b]

	Spraygel
Mann-Whitney U	4.000
Wilcoxon W	10.000
Z	-.221
Asymp. Sig. (2-tailed)	.825
Exact Sig. [2*(1-tailed Sig.)]	1.000[a]

a. Not corrected for ties.

b. Grouping Variable: Terhadap bakteri

Ranks

	Terhadap bakteri	N	Mean Rank	Sum of Ranks
Spraygel	S.aureus	3	3.67	11.00
	P.acnes	3	3.33	10.00
	Total	6		

Test Statistics[b]

	Spraygel
Mann-Whitney U	4.000
Wilcoxon W	10.000
Z	-.218
Asymp. Sig. (2-tailed)	.827
Exact Sig. [2*(1-tailed Sig.)]	1.000[a]

a. Not corrected for ties.

b. Grouping Variable: Terhadap bakteri

Ranks

	Terhadap bakteri	N	Mean Rank	Sum of Ranks
Spraygel	S.epidermidis	3	3.33	10.00
	P.acnes	3	3.67	11.00
	Total	6		

Test Statistics[b]

	Spraygel
Mann-Whitney U	4.000
Wilcoxon W	10.000
Z	-.232
Asymp. Sig. (2-tailed)	.817
Exact Sig. [2*(1-tailed Sig.)]	1.000[a]

a. Not corrected for ties.

b. Grouping Variable: Terhadap bakteri

Positive control

Ranks

	Terhadap bakteri	N	Mean Rank	Sum of Ranks
Kontrolpositif	S.aureus	3	5.00	15.00
	S.epidermidis	3	2.00	6.00
	Total	6		

Test Statistics[b]

	Kontrolpositif
Mann-Whitney U	.000
Wilcoxon W	6.000
Z	-2.023
Asymp. Sig. (2-tailed)	.043
Exact Sig. [2*(1-tailed Sig.)]	.100[a]

a. Not corrected for ties.

b. Grouping Variable: Terhadap bakteri

Ranks

	Terhadap bakteri	N	Mean Rank	Sum of Ranks
Kontrolpositif	S.aureus	3	4.17	12.50
	P.acnes	3	2.83	8.50
	Total	6		

Test Statistics[b]

	Kontrolpositif
Mann-Whitney U	2.500
Wilcoxon W	8.500
Z	-.913
Asymp. Sig. (2-tailed)	.361
Exact Sig. [2*(1-tailed Sig.)]	.400[a]

a. Not corrected for ties.

b. Grouping Variable: Terhadap bakteri

Ranks

	Terhadap bakteri	N	Mean Rank	Sum of Ranks
Kontrolpositif	S.epidermidis	3	2.00	6.00
	P.acnes	3	5.00	15.00
	Total	6		

Test Statistics[b]

	Kontrolpositif
Mann-Whitney U	.000
Wilcoxon W	6.000
Z	-2.023
Asymp. Sig. (2-tailed)	.043
Exact Sig. [2*(1-tailed Sig.)]	.100[a]

a. Not corrected for ties.

b. Grouping Variable: Terhadap bakteri

Negative control

Ranks

	Terhadap bakteri	N	Mean Rank	Sum of Ranks
Kontrolnegatif	S.aureus	3	3.50	10.50
	S.epidermidis	3	3.50	10.50
	Total	6		

Test Statistics[b]

	Kontrolnegatif
Mann-Whitney U	4.500
Wilcoxon W	10.500
Z	.000
Asymp. Sig. (2-tailed)	1.000
Exact Sig. [2*(1-tailed Sig.)]	1.000[a]

a. Not corrected for ties.

b. Grouping Variable: Terhadap bakteri

Ranks

	Terhadap bakteri	N	Mean Rank	Sum of Ranks
Kontrolnegatif	S.aureus	3	3.50	10.50
	P.acnes	3	3.50	10.50
	Total	6		

Test Statistics[b]

	Kontrolnegatif
Mann-Whitney U	4.500
Wilcoxon W	10.500
Z	.000
Asymp. Sig. (2-tailed)	1.000
Exact Sig. [2*(1-tailed Sig.)]	1.000[a]

a. Not corrected for ties.

b. Grouping Variable: Terhadap bakteri

Ranks

	Terhadap bakteri	N	Mean Rank	Sum of Ranks
Kontrolnegatif	S.epidermidis	3	3.50	10.50
	P.acnes	3	3.50	10.50
	Total	6		

Test Statistics[b]

	Kontrolnegatif
Mann-Whitney U	4.500
Wilcoxon W	10.500
Z	.000
Asymp. Sig. (2-tailed)	1.000
Exact Sig. [2*(1-tailed Sig.)]	1.000[a]

a. Not corrected for ties.

b. Grouping Variable: Terhadap bakteri