ANTIMICROBIAL EFFECTIVENESS OF APPLE CIDER VINEGAR IN THE GROWTH OF *STAPHYLOCOCCUS EPIDERMIDIS* AND *PROPIONIBACTERIUM ACNES*

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ABSTRACT

Acne is a skin disease caused by *Propionibacterium acnes*, *Staphylococcus epidermidis*-, and *Staphylococcus aureus*-induced inflammation. Apple cider vinegar contains antibacterial compounds such as acetic acid, chlorogenic acid, polyphenols, alkaloids, flavonoids, and glycosides. This study is conducted to determine the effectiveness of antibacterial compounds contained in apple cider vinegar against the growth of *S.epidermidis* and *P.acnes*, in vitro. The study used the disc diffusion method by utilizing the Mueller Hinton Agar medium. The apple cider vinegar inhibition zone was formed at the concentration of 25%, 50%, 75%, and 100% against the growth of *P.acnes*, whereas the inhibition zone against *S.epidermidis* was formed at the concentration of 50%, 75%, and 100%. The highest inhibition zone against P. acnes was 8,825 mm and against *S.epidermidis* was 3,725 mm at the concentration of 75%. Test results One-way ANOVA on *P.acnes* and Kruskal Wallis to *S.epidermidis* obtained (p) <0,005. This study concludes the effectiveness of apple cider vinegar in inhibiting the growth of *P. acnes* and *S. epidermidis*, where the effectiveness against *P. acnes* was stronger than against *S. epidermidis*.

Keywords: Apple cider vinegar; *Staphylococcus epidermidis*; *Propionibacterium acnes*.

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INTRODUCTION

Acne is an infectious disease that occurs on the skin and is a concern for young people. Acne occurs due to inflammation of the blocked polysebaceous glands with the characteristic presence of comedones, papules, pustules, and scars triggered by the bacteria *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* on the face, neck, upper arms, back, and chest (Roudhantini, 2013). *Propionibacterium acnes* and *Staphylococcus epidermidis* produce metabolites that react with sebum which will increase the occurrence of the inflammatory process so that acne is formed (Laianto, 2015).

Among the causes of acne, the bacterium *Propionibacterium acnes* is found in 68 – 79% of acne lesions with inflammation and is thought to play a major role in the development of acne by inducing the inflammatory process. *Propionibacterium acnes* is a gram-positive bacterium that dominates and forms colonies in the follicles of the sebaceous glands (Choi et al., 2018). *Propionibacterium acnes* will then cause moderate to severe inflammation in acne lesions, and increase the proliferation of keratinocyte follicles resulting in

hyperkeratinization which can create a better anaerobic environment for colonization of *Propionibacterium acnes* (Choi et al., 2018).

Some degree of acne affects almost all people aged 15-17 years with moderate to severe degrees of about 15-20%. Estimates of the prevalence of acne are difficult to compare because the definitions of acne and the severity of acne differ in different studies. Although acne is considered a disease in adolescents, it can continue into adulthood (Williams et al., 2012).

Apple cider vinegar is currently a topic that is often discussed as a traditional herbal remedy. The effects of apple cider vinegar have been studied for hundreds of years and were reported to have been used for the first time 5000 years ago in 400 BC, when Hippocrates, the father of modern medicine, formulated a mixture of honey and apple cider vinegar for use as a treatment for various ailments. During the American civil war, apple cider vinegar was used as an antiseptic to treat the wounds of soldiers (Gopal et al., 2017). Vinegar is generally made from fruits such as grapes, coconut, prunes, and tomatoes and can also be made from rice and potatoes, while apple cider vinegar is made from crushed apples and then bacteria and yeast are added to the liquid to start the alcoholic fermentation process. Sugar is converted to alcohol and in the next fermentation phase, alcohol is converted to vinegar by acetic acid-forming bacteria (Acetobacter) (Gopal et al., 2017).

Research conducted by Darshna Yagnik, Vlad Serafin, and Ajit J. Shah (2018) showed that apple cider vinegar has an antibacterial effect in inhibiting the growth of Escherichia coli, *Staphylococcus aureus*, and *Candida albicans*bacteria and also has an anti-inflammatory effect by inhibiting cytokines. cause of inflammation.

Kamal Putra (2016) conducted a study and gave results that Manalagi apple peel extract using ethanol solvent at concentrations of 30% and 50% had higher effectiveness as an antibacterial on Streptococcus agalactiae and Escherichia coli.

Based on the above background, it is necessary to conduct further research on apple cider vinegar with the topic of the effectiveness of apple cider vinegar as an antibacterial compound against *Staphylococcus epidermidis* and *Propionibacterium acnes* bacteria with different concentrations.

MATERIALS AND METHODS

This study aimed to determine the antibacterial effectiveness of apple cider vinegar in inhibiting the growth of *P.acnes* and *S.epidermidis* bacteria to several experimental groups, then the results were compared with the control group (Sastroasmoro S, 2011). The inhibitory power of apple cider vinegar was assessed by concentration (12 ,5%, 25%, 50%, 75%, 100%). Antibacterial effectiveness can be observed from the formation of an inhibition zone in the form of a clear area around the paper disc which is measured using a digital caliper.

a. Materials

This study used samples of apple cider This study used samples of apple cider vinegar obtained from supermarkets. The bacteria used were *Staphylococcus epidermidis* and *Propionibacterium acnes* obtained from microbiology department Faculty of Medicine UPN Veteran Jakarta which had been isolated and cultured on blood agar and Mueller Hinton Agar media.

The number of repetitions of each treatment group will be calculated using the Federer formula. The treatment group consisted of five concentrations of apple cider vinegar (12.5%, 25%, 50%, 75%, 100%), one negative control group (sterilised water for injection), and one positive control group (clindamycin antibiotic). Based on the calculation of the sample size that has been carried out by the author, so the minimum number of samples needed is 5 MHA (Mueller Hinton Agar) preparations that have been cultured with *S.epidermidis* and *P.acnes* bacteria for each experiment with 4 repetitions.

b. Method

This study used the diffusion method with disc paper. Filter paper discs, each containing different concentrations of apple cider vinegar, were placed on top of solid media that had been previously inoculated by *P.acnes* and *S.Epidermidis.* Furthermore, incubation was carried out using an incubator for 24 hours at 370 C, then the diameter of the inhibition zone formed around the paper disc was measured to measure the strength of the resistance against the test organisms (Hudzicki, 2010).

RESULT

In graph 1 it can be seen that apple cider vinegar with concentrations of 25%, 50%, 75%, and 100% showed the formation of inhibition zones, this indicates that apple cider vinegar with these concentrations has antibacterial effectiveness in inhibiting the growth of *Propionibacterium acnes*.

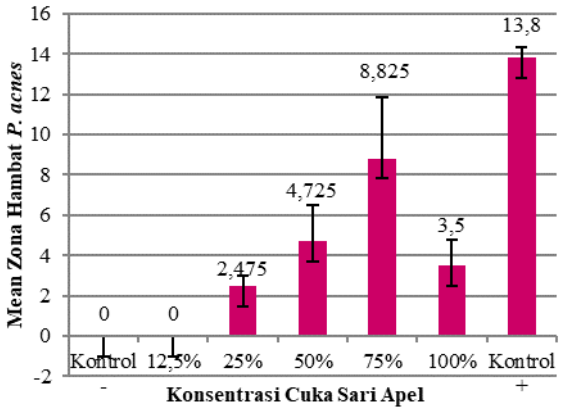


Figure 1 Results of Measurement of Inhibitory Zone Diameter Formed by Apple Cider Vinegar Against *Propionibacterium acnes*.

The concentration of 25% has shown the formation of an inhibition zone and the optimum inhibition zone is seen at a concentration of 75%. The negative control group did not show the formation of an inhibition zone which indicated that the negative control group had no effect in inhibiting the growth of *Propionibacterium acnes*. In the apple cider vinegar group, the inhibition zone formed was less effective when compared to the positive control, namely the antibiotic clindamycin.

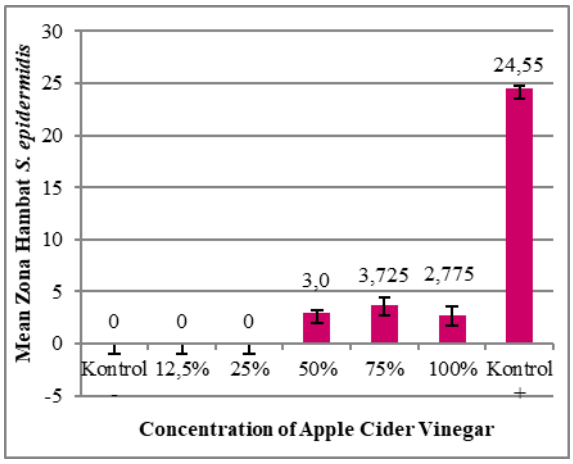
Table 1. Inhibition Zone Diameter of *Propionibacterium acnes* by Apple Cider Vinegar

Replications	Concentrations and control					
	12,5 %	25 %	50 %	75 %	100 %	Kontrol +
1	0	2,5	5,0	4,3	4,8	13,8
2	0	3,3	7,5	12,8	2,5	14,6
3	0	2,0	2,9	9,7	4,7	13,8
4	0	2,1	3,5	8,5	2,0	13,0
Mean	0	2,47	4,72	8,82	3,50	13,80
Deviation Standard	0	0,51	1,77	3,04	1,26	0,565

In graphs 1 and table 1, it can be seen that the group with the largest diameter of the inhibition zone was the positive control with a diameter of 13.8 mm, then the second-largest group was apple cider vinegar with a concentration of 75% with a diameter of 8.825 mm, then the third-largest was with apple cider vinegar with a concentration of 75%. 50% with an inhibition zone diameter of 4.725 mm, followed by a concentration of 100% with a diameter of 3.5 mm, and the one with the smallest inhibition was apple cider vinegar with a concentration of 25%. Apple cider vinegar with a concentration of 12.5% and a negative control did not show the formation of an inhibition zone.

Researchers did not use an oxygen absorber in this study so that the standard deviation formed in the 75% concentration of apple cider vinegar treatment against *Propionibacterium acnes* reached 3,047, which means that the sample data is more spread out or varies from the average. The standard deviation number can also be used to assess the difference between sample values and the average value of all data and can be used to assess the accuracy of the average in representing the values of all data. The high value of the standard deviation contained in the study can indicate that the resulting average value cannot represent the value of all data.

Based on the criteria for antibacterial inhibition according to Davis and Stout (1971) in the results of the research conducted (graph 1), it was found that apple cider vinegar with a concentration of 75% had moderate inhibitory power as an antibacterial and had a weak inhibitory power as an antibacterial at a concentration of 25%, 50 %, and 100%.



Graph 2 Results of Measurement of Inhibitory Zone Diameter Formed by Apple Cider Vinegar Against *Staphylococcus epidermidis.*

Figure 2 shows that there is an inhibition zone formed by giving apple cider vinegar with concentrations of 50%, 75%, and 100%, this indicates that apple cider vinegar with these concentrations has antibacterial effectiveness in inhibiting the growth of *Staphylococcus epidermidis* bacteria. New inhibition zones began to form on the administration of apple cider vinegar with a concentration of 50% and showed the optimum inhibition zone at a concentration of 75%. The negative control group showed no inhibition zone was formed which indicated that the negative control group could not inhibit the growth of *Staphylococcus epidermidis* bacteria. In the apple cider vinegar group, the inhibition zone formed was very effective when compared to the positive control, namely the antibiotic clindamycin.

Table 2. Diameter of Inhibitory Zone of *Staphylococcus epidermidis* by Apple Cider Vinegar

Replications	Concentrations and controls					
	12,5 %	25 %	50 %	75 %	100 %	Control +
1	0	0	2,8	3,1	2,0	24,6
2	0	0	2,8	3,4	2,6	24,2
3	0	0	3,2	4,9	4,2	24,8
4	0	0	3,2	3,5	2,3	24,6
Mean	0	0	3,0	3,72	2,77	24,55
Deviation Standard	0	0	0,2	0,69	0,84	0,217

In graphs 2 and table 2, it can be seen that the group with the largest diameter of the inhibition zone was the positive control with a diameter of 24.6 mm, much higher than the group receiving apple cider vinegar with various concentrations. At a concentration of 50%, the diameter of the inhibition zone has an average of 3.0 mm, while at a concentration of 75% the diameter of the inhibition zone has an average of 3.725 mm, and the smallest average diameter of the inhibition zone is found at 100% concentration which is 2.775. Apple cider vinegar with concentrations of 12.5%, 25%, and negative control showed no inhibition zone was formed.

According to the criteria for antibacterial inhibition of Davis and Stout (2009, p.667) in the results of the research conducted (graph 2), it was found that apple cider vinegar with concentrations of 50%, 75%, and 100% had weak inhibitory powers as antibacterial, while in the control Positive results found that the antibiotic clindamycin has a very strong inhibitory power as an antibacterial.

DISCUSSION

This study showed that the antibacterial effectiveness of apple cider vinegar against *Propionibacterium acnes* began to appear at a

concentration of 25%, while *Staphylococcus epidermidis* only started to appear at a concentration of 50%. The greatest inhibition of *Propionibacterium acnes* and *Staphylococcus epidermidis* was found at a concentration of 75%. In data processing using the One-way ANOVA test on *Propionibacterium acnes* bacteria and the Kruskal-Wallis test on *Staphylococcus epidermidis* both have a significance value <0.05 which indicates there is a significant difference in the treatment results between groups of apple cider vinegar concentrations. The results of data processing with the Post Hoc test of the antibacterial effectiveness of apple cider vinegar with various concentrations against *Propionibacterium acnes* found no significant difference (> 0.05), except in the comparison group between negative control, 12.5% concentration, 25% concentration, 50% concentration. , and a concentration of 100% with a positive control showing a significance value of less than 0.05 which means that there is a significant difference in the results of the apple cider vinegar treatment between these comparisons and the growth of *Propionibacterium acnes* bacteria. Meanwhile, in data processing using the Mann-Whitney test, the effectiveness of antibacterial against *Staphylococcus epidermidis* had a significant difference (<0.05), except at concentrations of 50% with 75% and 100%, and between concentrations of 75% and 100% showed a significance value > 0. 0.05, which indicates that there was no significant difference in the results of the apple cider vinegar treatment between the concentration ratio and the growth of *Staphylococcus epidermidis* bacteria.

Based on the research conducted, the increase in inhibition formed was seen along with the increase in the concentration of apple cider vinegar used. This can be seen from the average value that has increased in the diameter of the inhibition zone formed with each increase in the concentration of apple cider vinegar used. The increase in the inhibition zone formed was due to the concentration of the extract affecting the absorption of antibacterial compounds. The higher the concentration of the extract used, the more antibacterial compounds contained in it. This will increase the effectiveness of antibacterial in inhibiting bacterial growth which can be seen from the wide diameter of the inhibition zone formed (Munfaati et al., 2015, p.69).

At a concentration of 75% apple cider vinegar produced the largest average diameter of the inhibition zone for the two bacteria tested. However, when tested with 100% concentration of apple cider vinegar, the average inhibition zone value decreased. Based on these results, it is known that the most effective concentration of apple cider vinegar in inhibiting the bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis* is a concentration of 75%. At a concentration of 75% apple cider vinegar the high content of active compounds can be optimally absorbed into the paper disc and agar media, causing the diameter of the resulting inhibition zone to be large. Meanwhile, apple cider vinegar concentration of 100% is the maximum concentration, so it has very concentrated properties and concentration, which causes the diffusion process on disc paper and media to be less effective. This is what causes the inhibition zone that is formed to be smaller (Hidayati, 2017).

The concentration in question is the concentration of apple cider vinegar using a solvent called Sterilized Water For Injection. The reason for using the Sterilized Water For Injection solvent is that the Sterilized Water For Injection solvent reacts neutrally to the solute, namely apple cider vinegar and can avoid biased research results due to contamination from other bacteria.

This study provides results by research conducted by Kanzul Kamal Putra, Endang Setyowati, and Tri Eko Susilorini (2016) using *Streptococcus agalactiae* and *Escherichia coli* bacteria with concentrations of Manalagi apple peel extract 10%, 30%, and 50% with ethanol solvent 96 % as well as iodin solution as a positive control, where the bacteria used in this study were also Gram positive bacteria such as *Streptococcus*

agalactiae bacteria as was done by Kanzul Kamal Putra, Endang Setyowati, and Tri Eko Susilorini (2016) so that the results obtained were the same, namely The inhibition zone formed increased along with the increase in the concentration of Manalagi apple peel extract given.

The study also gave results that are by the research of Darsha Yagnik, Vlad Serafin, and Ajit J. Shah (2018) using *Escherichia coli* bacteria, *Staphylococcus aureus*, and the fungus *Candida albicans* with concentrations of 10%, 50%, 100% determining an increase in the inhibition zone. formed with increasing concentration of apple cider vinegar. Inhibition zones began to form in the research that the researchers had done which had the same results as the research of Darsha Yagnik, Vlad Serafin, and Ajit J. Shah, namely at a concentration of 50% apple cider vinegar on *Staphylococcus aureus* bacteria.

Research conducted by Gopal et al. (2017) with apple cider vinegar concentrations of 1%, 10%, 25%, 50%, and 100% gave the results that absolute inhibition could only be formed in apple cider vinegar with a concentration of 25% on bacteria *B.subtilis, E.coli,* and *P.auruginosa,* while the absolute inhibition zone formed on *S.aureus* bacteria was found in apple cider vinegar at a concentration of more than equal to 50% according to the results of the research that the researchers did because the bacteria used had the same characteristic which is gram-positive bacterium.

The difference between the previous research and the current study is that in this study the researchers used apple cider vinegar concentrations of 12.5%, 25%, 50%, 75%, and 100% because the bacterial species were different from previous studies so that the possible inhibition zones were formed is also different. The reason for the concentration of 12.5% was used in this study because in previous studies at a concentration of 10% no inhibition zone was found, while the reason for choosing a concentration of 75% in this study was because in previous studies the concentration used was too large, so the researcher in this study used an additional concentration of 75% concentration to determine the inhibition zone formed at that concentration. The research method used in this study is the disc diffusion method, the results obtained in this study are apple cider vinegar produces a larger diameter of inhibition zone in *Propionibacterium acnes* bacteria than *Staphylococcus epidermidis* based on calculations performed using a digital caliper.

Inhibition zones can be formed due to the presence of compounds that can interfere with bacterial growth such as acetic acid, chlorogenic acid, polyphenols, alkaloids, and flavonoids. Acetic acid is antibacterial by influencing the environment around the growth of bacteria to become acidic (Pratama et al., 2015). Chlorogenic acid by inhibiting enzymes involved in bacterial fatty acid synthesis can cause leakage of protoplasmic contents. (Kamal et al., 2016). Polyphenols by damaging cell membranes, destroy substrates, and disrupt the function of bacterial enzymes (Kamal et al., 2016). The mechanism of alkaloids as an antibacterial is by interfering with the components that make up the peptidoglycan of bacterial cells, then it will result in the layers contained in the bacterial cell walls not being completely formed so that bacterial cell death occurs (Darsana et al., 2012). The mechanism of flavonoids as an antibacterial is by disrupting the function of the bacterial cytoplasmic membrane and through interactions with bacterial DNA (Santosaningsih, 2011).

Staphylococcus epidermidis produces a type of toxin and a kind of mucus that makes it easy for it to stick in many places, including on surfaces made of glass or plastic. This mucus also causes *Staphylococcus epidermidis* to be more resistant to phagocytosis and certain antibiotics (Sinaga, 2004). This causes the inhibition zone of *Staphylococcus epidermidis* formed in this study to be smaller than the inhibition zone formed in *Propionibacterium acnes*.

Examination of the preservative content in the samples used in this study did not show the presence of sodium benzoate as a food preservative. The presence of preservative

content can be a confounding variable because it can give biased results in the research conducted, because the results showed negative in the apple cider vinegar sample used, it can be stated that the antibacterial effect produced was purely due to the antibacterial compounds contained in apple cider vinegar. used by researchers.

CONCLUSION

Based on the results of research and discussion, it can be concluded that apple cider vinegar has an antibacterial effect on the growth of *Propionibacterium acnes* and *Staphylococcus epidermidis* bacteria in vitro. epidermidis, and the most effective concentration of apple cider vinegar in inhibiting the growth of *Propionibacterium acnes* and *Staphylococcus epidermidis* bacteria was at a concentration of 75%.

REFERENCES

Choi, EJ, Lee, HG, Bae, IH, Kim, W, Park, J, Lee, TR, Cho, EG 2018, '*Propionibacterium acnes*-Derived Extracellular Vesicles Promote Acne-Like Phenotypes in Human Epidermis', *Journal of Investigative Dermatology*. *Society for Investigative Dermatology*, accessed on July 2018. https://www.ncbi.nlm.nih.gov/pubmed/29409885

Darsana, IGO, Besung, INK, Mahatmi, H 2012, 'Potensi Daun Binahong (*Anredera Cordifolia (Tenore) Steenis*) dalam Menghambat Pertumbuhan Bakteri *Escherichia coli* secara In Vitro', *Indonesia Medicus Veterinus*, accessed on October 2018. https://ojs.unud.ac.id/index.php/imv/article/view/1879

Davis, WW & Stout, TR 1971, 'Disc Plate Method of Microbiological Antibiotic Assay'. *Applied and Enviromental Microbiology*, accessed on October 2018. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC376382/

Gopal, J, Anthonydhason, V, Muthu, M, Gansukh, E, Jung, S, Iyyakkannu, S 2017, 'Authenticating apple cider vinegar's home remedy claims: antibacterial, antifungal, antiviral properties and cytotoxicity aspect', *Natural Product Research*, accessed on December 2018. https://www.ncbi.nlm.nih.gov/pubmed/29224370

Hidayati, Irma Rizki 2017, 'Pengaruh Ekstrak Daun Tembakau (*Nicotiana tabacum L.*) Sebagai Antimikroba Terhadap *Pseudomonas aeruginosa* ATCC 27853 dan *Escherichia coli* ATCC 25922 Secara *In Vitro',* Fakultas Kedokteran Universitas Pembangunan Nasional "Veteran" Jakarta, Jakarta

Hudzicki, J 2010, *Kirby-Bauer Disk Diffussion Susceptibility Test Protocol*, accessed on 07 June 2018. http://www.microbelibrary.org/index.php/library/laboratory-test/3189 kirby-beauer-disk-diffusion-susceptibility-test-protocol/

Laianto, S 2015, 'Uji Efektivitas Sediaan Gel Anti Jerawat Ekstrak Etanol Buah Pare (Momordica charantia) Terhadap *Staphylococcus epidermidis* dan *Propionibacterium acnes* Dengan Metode Difusi', accessed on October 2018. http://jurnal.untan.ac.id/index.php/jmfarmasi/article/view/846

Munfaati, PN, Ratnasari, E, Trimulyono, G 2015, 'Aktivitas Senyawa Antibakteri Ekstrak Herba Meniran (Phyllanthus Niruri) Terhadap Pertumbuhan Bakteri Shigella dysentriae Secara In Vitro', LenteraBio, accessed on February 2019.jurnalmahasiswa.unesa.ac.id/article,pdf

Pratama, RI, Husin, UA, Trusda, SAD 2015, 'Efek Antibakteri Cuka Sari Apel Terhadap *Salmonella Typhi*', accessed on August 2018. http://karyailmiah.unisba.ac.id/index.php/dokter/article/view/1337

Putra, KK, Setyowati, E, Susilorini, TE 2016, 'Inhibition of *Malus sylvestris Mill*. Peelextract Using Etanol Solvent On The Growth Of *Streptococcus*

agalactiae and *Escherichia coli* Causing Mastitis', *J. Ternak Tropika*, accessed on September 2018. https://ternaktropika.ub.ac.id/index.php/tropika/article/view/273

Roudhantini 2013 'Uji Efektivitas Sediaan Gel Anti Jerawat Minyak Atsiri Daun Jeruk Sambal (X Citrofortunella microcarpa (Bunge) Wijnands) Terhadap *Propionibacterium acnes* dan *Staphylococcus epidermidis'*, accessed on August 2018. http://jurnal.untan.ac.id/index.php/jmfarmasi/article/view/3984

Santosaningsih, D & Roekistiningsih 2011, 'Efek Ekstrak Daun Salam (Eugenia polyantha Wight) terhadap Penghambatan Pembentukkan Biofilm pada *Staphylococcus aureus* secara in vitro', Fakultas Kedokteran, Universitas Brawijaya, Malang.

Sinaga, E 2004, Infeksi Nosokomial dan *Staphylococcus epidermidis*, EGC, Jakarta. p. 146-154.

Williams, HC, Dellavalle, RP, Garner, S 2012, 'Acne Vulgaris', accessed on July 2018. https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(11)60321-8/fulltext