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IN VITRO ANTIBACTERIAL EFFECTIVENESS TEST OF SEVERAL HERBAL PLANT EXTRACT IN AN ATTEMPT TO DISCOVER THE STRONGEST ANTIBACTERIAL HERBAL TOPICAL AGAINST *STAPHYLOCOCUS AUREUS* DAN *PSEUDOMONAS AERUGINOSAE*

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ABSTRACT
Researchers have tested the activity of the water phase ointment and the cork fish oil extract phase in acute stage II open wounds. The results showed a significant healing potency towards negative controls. However, it does not provide optimal results because a crust was formed. Therefore, researchers used a closed system (dressing) that is able to eliminate crust. Based on the research conducted, crust was not form in the activity test done by researcher using cork fish ointment with male white rats (*Rattus norvegicus*) wistar strain. But there is a new problem arise such as infection. Based on these results, the researchers aim to look for herbal extracts that can be used as the most powerful antibacterial that will be use in topical wound drug formulation and be tested in vivo to see the effect of wound healing towards white wistar strain rats. This will then be further develop into topical wound drug product. Researchers have conducted in vitro literature studies on several plants, discovered approximately 18 plant extracts that have antibacterial benefits. Antimicrobial activity was measured in vitro by the Kirby Bauer method, which use Mueller-Hinton Agar (MHA) as a bacterial growth medium. There are two bacteria used, namely *Staphylococus aureus* and *Pseudomonas aeruginosa*, where each bacterium is tested thrice on the extract of each plant with a bacterial suspense concentration of 1.5 x 108 cells / mL. Positive control using ciprofloxacin injection of 2 mg / mL. Inhibition zone formed is measured using a calipers (caliver). The inhibitory zones produced in each plant extract were then compared with each other. The data obtained will be displayed in the form of descriptive statistics. Sample with largest inhibitory zone respectively are clove oil and green betel extract, with significantly different significance value compared to other plant extract.

KEYWORDS: Antibacterial, topical, herbal plant extract, in vitro.

INTRODUCTION
Antibacterial is a compound used to control the growth of harmful bacteria. Controlling the growth of microorganisms aims to prevent the spread of disease and infection, eradicate microorganisms in the infected host, and to prevent spoilage and destruction of material by microorganisms.[1] Bacterial resistance against antibiotics is one of worldwide problems in both developed and developing countries. The treatment of S. aureus infections has become more complex due to the emergence of various types of antibiotic resistance throughout the world. So far, drugs circulate on market tend to rely on the use of antibiotics to speed up the process of wound healing, such as in the form of topical preparations that contain antibiotics like neomycin sulfate. This can trigger bacterial resistance. Based on these facts, it can be concluded that treatment using antibiotics can trigger bacterial resistance, so researchers feel it is important to conduct a research on topical drugs for wound healing in the presence of active substances derived from natural ingredients that are safer and more effective.

A research on cork fish oil ointment was conducted with a closed system using dressings in male white rats (*Rattus norvegicus*) wistar strain. From this research, it is proven that there is no formation of crusting. But there is a new problem such as infection identified from festering wound, slough, and smell. Based on the results of the research that has been done, the researchers aim to look for herbal extracts that can be used as the most powerful antibacterial of medicinal plants include green betel leaf extract (*Piper betle* L.), clove oil (*Syzygium aromaticum*, L.), and kelulut honey (*Trigona* Sp.), Red betel (*Piper betle* Linn.), Onion (*Allium ascalonicum* L .), garlic (*Allium sativum* L.), red ginger (*Zingiber officinale*), white ginger (*Zingiber officinale*), turmeric (*Curcuma domestica* Val), kratom (*Mitragina Speciosa*), gotu kola

(*Centella asiatica* L.), kluwak (*Pangium edule*), binahong (*Anredera cordifolia*), fruit and leaves of starfruit (*Averrhoa bilimbi* L.), fruit and leaf of noni (*Morinda citrifolia* L), and virgin coconut oil (*Virgin Coconut Oil*).

Betel leaf (*Piper betle* L.) contains essential oils consisting of phenol compounds and some derivatives of euganol and cavikol. Phenol compounds and their derivatives can denaturate bacterial cell proteins. Euganol compounds are bactericidal by increasing bacterial permeability. Clove oil is a broad-spectrum antibacterial that can inhibit the growth of bacteria *B. subtilis, B. cereus, E.coli, P. aeruginosa* and *S. Aureus*.[2] Honey can be used for wounds, namely as an antibacterial and can accelerate tissue growth in wounds[3], the outer layer of onions that are dry and often brown in color are rich in fiber and flavonoids as well as antibacteria agent against *Stapylococcus aureus* and *E. coli*[4], Bioactive substances that act as antibacterial in garlic are volatile allicin with sulfur content[5], Red ginger extract (*Zingiber officinale var. Rubrum*) has an antibacterial effect against Gram-positive and Gram-negative bacteria.[6] The content of ginger has an effect on bacteria. The content that has been studied and is known to be responsible for the antibacterial effect is terpene compounds. In vitro, proving that the active compounds in turmeric are able to inhibit the growth of fungi, viruses, and bacteria both Gram positive and Gram negative, such as *E. coli* and *Staphylococcus aureus*, because turmeric contains various compounds including curcumin and essential oils[7], Kratom leaf methanol extract (*Mitragyna speciosa* Korth.) has antibacterial activity against bacteria *Escherichia coli* and *Staphylococcus aureus*, gotu kola content has functions as antibacterial, such as saponins. According to the research of J. Barnes et al in 1996 that the saponin derivative, asiaticoside is lipophilic and can form complex compounds with cell membranes through hydrogen bonds, then destroy the permeability of bacterial cell walls. Concentration of 3% (w / v) of picung seeds has inhibited the growth of gram-positive stem bacteria (*Bacillus* sp.), Gram-positive coccus (Micrococcus sp.), Gram-negative non-fermentative stem-negative bacteria (*Pseudomonas* sp.) And gram-negative fermentative stem-negative bacteria (*coliform*), Noni has antibacterial (phenol), antiviral, antifungal, antitumor, antihelmic, analgesic, hypotensive, anti-inflammatory, and immune enhancing effects.[8] Honey is usually used for health food, medicine and cosmetics. Evidence shows that honey can be use for wound as an antibacterial and could speed up the growth of wound tissue.[3] Independent lauric acid also have antibacterial activity, such as lauric acid as well as monolaurin in virgin coconut oil having antibacterial activity against *S. Aureus*.

MATERIALS DAN METHOD

Tools

Tools used for extraction are aluminium foil, blender (Toshiba), horn spoons, filter paper, vacuum rotary evaporator, and waterbath. Extract standardization tools are analytical scales ((Precisatipe XB 4200C)), stainless spoon, oven (Memmert UP400), porcelain crucible and desiccators. The tools used for antibacterial testing are petri dishes, shakers, test tubes, filter paper, cotton wool, media bottles, ose needles, autoclaves, incubators, tweezers, bunsen, micro pipettes, tissues, calipers and rulers.

Materials

Materials in making extract such as medicinal plant simplisia extract are betel leaf extract (*Piper betle* L.), clove oil (*Syzygium aromaticum* L.), dan kelulut honey (*Trigona* Sp.), red betel leaf (*Piper betle* L.), shallots (*Allium ascalonicum* L.), garlic (*Allium sativum* L.), red ginger (*Zingiber officinale*), white ginger (*Zingiber officinale*), turmeric (*Curcuma domestica Val*), kratom (*Mitragina speciosa*), centella (*Centella asiatica* L.), kluwak (*Pangium edule*), binahong (*Anredera cordifolia*), starfruit fruit and leaves (*Averrhoa bilimbi* L.), noni fruit and leaves (*Morinda citrifolia* L), and coconut oit (*Virgin Coconut Oil*), DMSO, aquadest. Material used in antibacterial are ciprofloxacin injection, Mueller Hinton Agar medium, *Staphylococus aureus*, and *Pseudomonas aeruginosa* bacterial suspense.

Ways of Working

1. Sample Preparation

Samples in the form of leaf were carried out in the morning. Green betel (*Piper betle* L.), clove oil (*Syzygium aromaticum*, L.), and kelulut honey (*Trigona* Sp.), red betel (*Piper betle* L), onion (*Allium ascalonicum* L.), garlic (*Allium sativum*) L.), red ginger (*Zingiber officinale*), white ginger (*Zingiber officinale*), turmeric (*Curcuma domestica* Val), kratom (*Mitragina Speciosa*), gotu kola (*Centella asiatica* L.), kluwak (*Pangium edule*), binahong (*Anredera cordifolia*), starfruit fruit and leaves (*Averrhoa bilimbi* L.), noni fruit and leaves (*Morinda citrifolia* L), and virgin coconut oil (*Virgin Coconut Oil*), which are taken must be selected according to the criteria of fresh leaves or fruit, not deformed, picked manually (using hands) and sort out in wet condition for extraction. Material processing is done by washing the samples with clean and running water. Then the leaves are dried in the sun with a black cloth covered for 3-4 days, then the dried simplisia is made to rough powder with a blender, then stored in a closed container. Dried simplisia leaf powder will be used to make extracts. Clove oil (USFI) is obtained at the drugstore in KH. Wahid Hasyim street. Kelulut honey (Trigona Sp) is obtained at Kitamura Clinic KH. Wahid Hasyim street, number.44, Pontianak City, West Kalimantan. Indonesia.

2. Biology Determination

Sample used is determinated in Biology laboratorium, Biology department, Matematics and Natural Science Faculty, Tanjungpura University, Pontianak, West Kalimantan.

3. DMSO Extract Production

Extraction is done by maceration. Simplicia is stored in a glass container. Then, poured and soaked by DMSO 10-20 times from the amount of sample. The glass container was closed and stay put for 24 hours while be stirred occasionally. The maserate obtained was then stored in a glass bottle, and was macerated again. The result of maceration will be collected and filtered. Maserate will be concentrated using *rotary evaporator*,and finally concentrated once again by aerated them in room temperature until it become thick extract.

4. In Vitro Test

In vitro tests were carried out at the Biology Laboratory, Faculty of Pharmacy, Tanjungpura University, Pontianak, West Kalimantan. The working procedure include are first making bacteria culture on the Nutrient Broth media that was evenly distributed on the surface of the Mueller Hinton Agar (MHA) media, and was left for 5 minutes. The number of bacteria is in accordance with Mc Farland 3 standard (± 9x108 / ml). Empty disc paper that has been soaked in DMSO extract samples and stew samples, then were placed in a sterile petri dish for 5 minutes until no liquid drops. Then the disc paper is placed on the surface of the MHA media pressed slightly so that it sticks. As a positive control, 5 µg ciprofloxacin antibiotic disk paper was used. Then the MHA media was incubated at 37 ° C for 24 hours. Inhibitory zone area is formed and is measured by the calipers (caliver).

5. Data Analysis

The result obtained from the research is the inhibitory zone that was formed. The mean value of inhibitory zone will then be known. Beside that, the inhibitory zone formed can be observed to know it's effectiveness against positive gram bacteria or negative gram bacteria. The result obtained will be displayed in descriptive statistic.

RESULTS AND DISCUSSION

The research done is to test the antibacterial effectiveness in some plant extract (sample). Antibacterial effectiveness in it will be tested in vitro. Kirby Bauer metode will be used to identify the presence or absence of inhibitory zone or clear zone formed in the media by using calipers (caliver).

Every plant extract was treated by each bacteria (*Staphylococcus aureus*, and *Pseudomonas aeruginosa*) in Mueller Hinton Agar (MHA) media. *Staphylococcus aureus* is a round Gram positive bacteria with 0,7 1,2 µm diameter, it is arranged in an irregular group like grape shape, facultative anaerobes, does not form spores, and does not move. This bacteria will grow in optimum temperature at 37 ºC, but form the best pigment at room temperature (20-25 ºC). *Pseudomonas aeruginosa* bacteria is rod-shaped, motile and is about 0,6 x 2 mm big. Gram negative bacteria can appear individually, paired or sometimes in the form of short chain. *P.aeruginosa* can grow very well at 37–42°C. *Pseudomonas aeruginosa* bacteria cannot be cured by single therapy because of the low success rate and the bacteria will become quickly resistant. The use of *Staphylococcus aureus* (positive gram bacteria), and *Pseudomonas aeruginosa* (negative gram bacteria) is aim to see how broad is the spectrum of antibacterial effectiveness formed by medicinal extract. Each treatment will be done thrice. This repetition aims to ensure the validity of the experimental results.

No	Sample	Inhibitory Zone (mm)							
		Staphylococcus Aureus			Mean	Pseudomonas Aeruginosa			Mean
		1	2	3		1	2	3	
1	Minyak Cengkeh (MC)	18,1	13,7	15,05	15,6	22,2	17,9	15,7	18,6
2	VCO	7,2	8,25	7,3	7,6	9,8	9,1	9,4	9,4
3	Kratom (KR)	7,3	9,4	7,65	8,1	9,8	11,3	12	11
4	Pegagan (PG)	7,05	7,4	6,4	7	7,3	6,85	7,5	7,2
5	Biji Kluwak (KL)	7,2	7,3	8,5	7,7	9,95	7,35	8	8,43
6	Binahong (BN)	7,45	7,85	8,95	8,1	7,15	7,2	6,65	7
7	Belimbing Wuluh (BW)	8,8	8,5	9,85	9,1	8,95	7,3	9,3	8,5
8	Daun Belimbing Wuluh (DBW)	7,1	7,4	6,9	7,1	8,6	7,6	7,6	7,93
9	Daun Sirih Hijau (SH)	13,7	14,1	14,8	14,2	12,15	11,5	10,1	11,25
10	Daun Sirih Merah (SM)	7,45	8,55	9,05	8,4	8,45	9,2	8,25	8,6
11	Daun Mengkudu (DM)	8,8	8,9	7,7	8,5	7,45	8,85	7,15	7,8
12	Buah Mengkudu (BMK)	7,4	8,45	8,45	8,1	6,8	7,6	8,7	7,7
13	Bawang Merah (BM)	0	7,15	0	2,4	6,65	7,55	7,6	7,3
14	Bawang Putih (BP)	0	6,9	7,85	4,9	7,05	7,45	7,6	7,4
15	Jahe Merah (JM)	7,5	9,3	8,8	8,5	9	8,3	9,95	9,1
16	Jahe Putih (JO)	7,45	7,9	8,1	7,8	8,55	8,15	8,25	8,3

17	Madu Kelulut (MD)	9,65	0	0	3,2	10,5	7,55	6	8,0
18	Kunyit (KUN)	11,45	8,05	13,85	11,1	8,6	8,95	9,8	9,1
19	Ciprofloxacin	32,95	33,15	31,2	32,4	29,1	26,85	27,25	27,7
20	DMSO	0	0	0	0	0	0	0	0

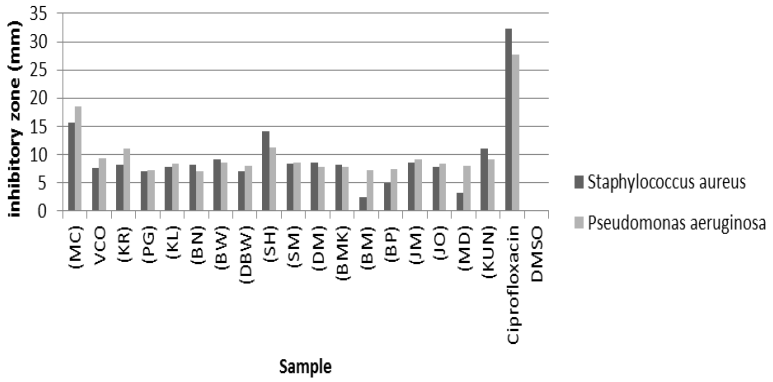
The results from the measurement of clove oil inhibition zone against *Staphylococcus aureus* bacteria obtained an average of 15.6 mm, and against *Pseudomonas aeruginosa* bacteria obtained an average of 18.6 mm. The results from the measurement of the inhibition zone of virgin coconut oil (*Virgin Coconut Oil*) against *Staphylococcus aureus* bacteria obtained an average of 7.6 mm, and the bacteria of *Pseudomonas aeruginosa* obtained an average of 9.4 mm. The results from the measurement of inhibition zone of kratom extract (*Mitragina speciosa*) on *Staphylococcus aureus* bacteria were found to be averagely at 8.1 mm, as well as on the *Pseudomonas aeruginosa* bacteria obtained at an average of 11 mm. The results of the inhibition zone measurement of *Centella asiatica L*. on *Staphylococcus aureus* bacteria obtained an average of 7 mm, and the bacteria of *Pseudomonas aeruginosa* obtained an average of 7.2 mm. The results of the inhibition zone measurement of kluwak (*Pangium edule*) seed extracts against *Staphylococcus aureus* bacteria were obtained at an average of 7.7 mm, as well as against the *Pseudomonas aeruginosa* bacteria which were found to be 8.43 mm. The results of the measurement of inhibition zone of binahong extract (*Anredera cordifolia*) on *Staphylococcus aureus* bacteria were found to be averagely at 8.1 mm, and on the bacteria of Pseudomonas aeruginosa the average was at 7 mm. The results of the measurement of inhibition zone of binahong extract (*Anredera cordifolia*) on *Staphylococcus aureus* bacteria were found to be averagely at 8.1 mm, and on the bacteria of *Pseudomonas aeruginosa* the average was at 7 mm. The results of the inhibitory zone extract of starfruit (*Averrhoa bilimbi L*.) extracts against *Staphylococcus aureus* bacteria obtained an average of 9.1 mm, and against the *Pseudomonas aeruginosa* bacteria an average of 8.5.

The results from the measurement of inhibition zones of starfruit leaf extract (*Averrhoa bilimbi L*.) against *Staphylococcus aureus* bacteria were obtained by an average of 7.1 mm, and against *Pseudomonas aeruginosa* bacteria obtained by an average of 7.93 mm. The results from the measurement of inhibition zones of green betel leaf extract (*Piper betle L*.) against *Staphylococcus aureus* bacteria were obtained an average of 14.2 mm, and the bacteria of *Pseudomonas aeruginosa* obtained an average of 11.25 mm. The results of the measurement of inhibition zones of red betel leaf extract (*Piper betle L*.) on *Staphylococcus aureus* bacteria were found to be an average of 8.4 mm, and on the bacteria of *Pseudomonas aeruginosa* on an average of 8.6 mm. The results from the measurement of inhibitory zones of Noni leaf extract (*Morinda citrifolia L*) against *Staphylococcus aureus* bacteria were obtained by an average of 8.5 mm, and against the *Pseudomonas aeruginosa* bacteria were obtained by an average of 7.8 mm. The results from the inhibition zone measurement of noni fruit extract (*Morinda citrifolia L*) against *Staphylococcus aureu*s bacteria were obtained at an average of 8.1 mm, and the bacteria of *Pseudomonas aeruginosa* obtained an average of 7.7 mm. The results from the measurement of inhibition zones of onion extract (*Allium ascalonicum L*.) on *Staphylococcus aureus* bacteria obtained an average of 2.4 mm, and on the bacteria *Pseudomonas aeruginosa* obtained an average of 7.3 mm. The results of the measurement of inhibition zones of garlic extract (*Allium sativum L.)* on *Staphylococcus aureus* bacteria obtained an average of 4.9 mm, and on the bacteria *Pseudomonas aeruginosa* obtained an average of 7.4 mm. The results of the measurement of inhibition zones of red ginger extract (*Zingiber officinale*) against *Staphylococcus aureus* bacteria were obtained an average of 8.5 mm, as well as against the *Pseudomonas aeruginosa* bacteria obtained an average of 9.1 mm. The results of the inhibition zone measurements of white ginger extract (*Zingiber officinale*) on *Staphylococcus aureus* bacteria were obtained at an average of 7.8 mm, as well as on the *Pseudomonas aeruginosa* bacteria obtained at an average of 8.3 mm. The results from the measurement of the inhibited zone of kelulut honey (*Trigona Sp.*) On the *Staphylococcus aureus* bacteria were found to be an average of 3.2 mm, and on the *Pseudomonas aeruginos*a bacteria an average of 8 mm was obtained. The results from the measurement of the inhibition zone of turmeric extract (*Curcuma domestica*) on the *Staphylococcus aureus* bacteria were found to be 11.1 mm, and on the *Pseudomonas aeruginosa* bacteria obtained an average of 9.1 mm.

The results from the measurement of the inhibition zone using positive control (Ciprofloxacin) on *Staphylococcus aureus* bacteria obtained an average of 32.4 mm, as well as on the *Pseudomonas aeruginosa* bacteria obtained an average of 27.7 mm. Ciprofloxacin mechanism of action as an antibacterial is by binding to the DNA gyrase enzyme subunit, and blocking the enzyme activity that is essential in maintaining supercoiling DNA and is important in the process of DNA replication. Mutations in the DNA girase coding gene cause the production of active enzymes, however, cannot be bound by fluoroquinolones. The results from the measurement of the positive control inhibition zone (Ciprofloxacin) show that the inhibition zone is greater than the inhibition zone produced by plant extracts. Plant extracts that have the most inhibitory zone values that approach the positive control are clove oil (Syzygium aromaticum, L.). Clove oil (Syzygium aromaticum, L.) has a large inhibitory zone value in both types of bacteria (Staphylococcus

aureus, and Pseudomonas aeruginosa), which shows clove oil has a broad spectrum antibacterial activity.

The results of the research showed that clove oil sample gave the largest inhibitory zone value compared to other plant extract samples. The statistical test results is determine using SPSS version 22. The normality test results indicate that the inhibitory zone values are normally distributed (p> 0.05). Furthermore, the Homogenity of Variances test was performed, it was found that the inhibitory zone values were homogeneous (p> 0.05). Data analysis was continued with parametric test namely One Way ANOVA because the data had fulfilled the requirements of normal distribution and homogeneity. Parametric test results showed that the data had significant differences between groups of 0,000 (p <0.05), this indicated that between the clove oil, betel green groups, positive control (ciprofloxacin), and negative control (DMSO) had different inhibitory zones significant. The inhibitory zone based on Towaha et al was caused by the high levels of eugenol contained in cloves as essential oils. The minimum content of eugenol compounds in clove oil according to SNI-06-2387-2006 is at least 78%. Thus it can be concluded that the extract of medicinal plants (green betel leaf extract (*Piper betle L.*), clove oil (*Syzygium aromaticum, L.*), kelulut honey (*Trigona Sp.*), Red betel (*Piper betle Linn.*), Red onion (*Allium ascalonicum L.*), garlic (*Allium sativum L.*), red ginger (*Zingiber officinale*), white ginger (*Zingiber officinale*), turmeric (*Curcuma domestica*), kratom (*Mitragina Speciosa*), gotu kola (*Centella asiatica L.),* kluwak (*Pangium edule*), binahong (*Anredera cordifolia*), fruit and leaves of starfruit *(Averrhoa bilimbi L.*), noni fruit and leaf (*Morinda citrifolia L*), and virgin coconut oil (*Virgin Coconut Oil*)) can inhibit the growth of *Staphylococcus aureus* bacteria, and *Pseudomonas aeruginosa*, but clove oil has better effectiveness.



Inhibitory Zone Diagram.

CONCLUSION

Every plant have antibacterial activity in in vitro test and sample with the largest inhibitory zone respectively are clove oil and green betel extract with the significantly different significance value compared to other plant extract. The result from the measurement of the inhibitory zone in clove oil against Staphylococcus aureus are in the average of 15,6 mm, as well as *Pseudomonas aeruginosa* bacteria in the average of 18,6 mm. The result from the measurement of the inhibitory zone in green betel against (*Piper betle* L.) Staphylococcus aureus are in the average of 14,2 mm, as well as *Pseudomonas aeruginosa* bacteria in the average of 11,25 mm.

SUGESTION

A further in vivo research for both clove oil and green betel extract sample is needed to identify the antibacterial effect to the wound directly.

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