NUSANTARA BIOSCIENCE
Vol. 9, No. 1, pp. 92-96
February 2017

ISSN: 2087-3948
E-ISSN: 2087-3956
DOI: 10.13057/nusbiosci/n090116

Antibacterial activity of ethanolic extracts of rhizome from three ginger varieties against acne-isolated bacteria

IDA INDRAWATI♥, MIA MIRANTI , ISY'AINI RIFQA MAYFI
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. Jl Raya Bandung-Sumedang KM 21, Jatinangor, Sumedang 45363, West Java, Indonesia. Tel./Fax.: +62 22-22-7796412, ♥email: ida.indrawati81@gmail.com

Manuscript received: 5 October 2016. Revision accepted: 20 February 2017.

Abstract. *Indrawati I, Miranti M, Mayfi IR. 2017. Antibacterial activity of rhizome extracts of three ginger varieties against acne-isolated bacteria. Nusantara Bioscience 9: xxxx.* Antibacterial activity of rhizome extracts of three ginger (*Zingiber officinale* Rosc.) varieties against bacteria isolated from acne was done. Three ginger varieties, namely emprit, gajah and red ginger, were tested to obtain the best concentration of ethanolic extracts among them in inhibiting the growth of the acne-isolated bacteria. This research used experimental approach by Completely Randomized Factorial Design with two factors of treatment, A x B. The first factor (A) were bacteria strain isolated from acne that consists of 3 levels. The second factor (B) was a varied concentration of rhizome ethanolic extract of three ginger varieties which consist of 7.5, 10, 12.5, 15, 17.5 and 20% for emprit and gajah, while for the concentration used for red were 2, 2.5, 5, 7.5, 10 and 12.5%. Moreover, a synthetic antibiotic clindamycin 30 mg mL^{-1} was used as a comparison. The obtained data were statistically analyzed using ANOVA with 95% of confidence and then followed by Tukey test when the significance was detected. The results showed that the acne-isolated bacteria were identified as *Propionibacterium* sp 1 and 2 and *Staphylococcus*. The rhizome ethanolic extract of red ginger had the highest antibacterial activity against the tested bacteria. This finding was supported by the fact that the red ginger variety had a small value of Minimum Inhibitory Concentration (MIC). Additionally, the measurement of clear zone revealed that the interaction between ethanolic extract of red ginger with a concentration of 12.5% producing a clear zone of 13.5, 25.0 and 17.5 mm against *Propionibacterium* sp.1, *Staphylococcus* sp and *Propionibacterium* sp.2, respectively.

Keywords: Acne, Antibacterial, clindamycin, *Propionibacterium, Staphylococcus, Zingiber officinale*

INTRODUCTION

Skin is the primary interface that has direct contact between the human body and the environment due to its position as the outermost layer of the body. Such skin-environment interactions make the skin susceptible to disease. Acne is one of the most prevalent skin problems and a chronic obstructive disease due to inflammatory in the pilosebaceous unit that is commonly found in adolescence individuals (Movita 2013). The main factors involved in the formation of acne are the growth of bacteria such as *Propionibacterium acnes*, *Staphylococcus epidermidis* or *Staphylococcus aureus*, that result in an increase in the production of sebum and inflammation (Tanghetti 2013). Many drugs were used to treat acne problems, such as azelaic acid, benzoyl peroxide, retinoids (Movita 2013) and also antibiotics such as clindamycin and tetracycline (Zu et al. 2012). However, these drugs can cause a variety of problems such as antibiotic resistance, irritation and immune hypersensitivity (Zu et al. 2012). A recent discovery has revealed the beneficial properties of natural drugs attracting the attention of many. The success of ginger in treating acne, for example, may be attributed to the fact that it contains antimicrobial substances such as zingiberol, zingiberene, and bisabolene. Moreover, the rhizome of ginger contains pungent vanillyl ketones such as gingerol and paradol. Gingerol is a mixture of crystal gingerone and source of acid that plays a significant role in inhibiting bacteria such as *S. aureus*, *Trichomonas vasmalis* (Derrida 1999). Another similar study also found that gingerol helps to cure bacterial vaginosis and skin diseases.

Ginger (*Zingiber officinale* Rosc.), a member of Zingiberaceae family, has been very popular to be used not only as spices but also as traditional plant medicine used to treat cough, diarrhea and bacterial infections. Differentiated by their shape, color and size of their rhizomes, three varieties of ginger are known, namely the emprit ginger (*Zingiber officinale* var. amarum), gajah ginger (*Zingiber officinale* var. officinale) and red ginger (*Zingiber officinale* var. rubrum). These three varieties of ginger have different chemical constituent of volatile oils (Setyawan 2002). In general, secondary metabolites contained in the ginger are a flavonoids, phenol, terpenoids and essential oils. These secondary metabolites can inhibit the growth of pathogens including *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus* sp. (Tanghetti 2013). Ginger extracts possess several medicinal properties and antibacterial activities, in which the inhibition of bacterial growth was dose dependent. This result may suggest that the n-hexane, ethyl acetate and soxhlet from ginger roots extracts could potentially be used to treat bacterial infections, dyspepsia, and colic. These extracts may also be used for treating the common cold, digestive disorders, hypercholesterolemia, heart diseases, lung diseases and could also be used as an

analgesic, particularly, in relieving pains from arthritis (Malu et al. 2009).

The common method used to obtain the active substances from the plant is by using an appropriate solvent which shares polarity or known as extraction. One of the most frequently used methods for the extraction is maceration. Based on the previous study upon the antibacterial activity of ethanol extracts, ginger potentially inhibits the growth of acne-origin bacteria.

The purpose of this research was to obtain the best concentration of ginger ethanolic extracts among three tested varieties in inhibiting the growth of acne-origin bacteria.

MATERIALS AND METHODS

Materials

Materials used in this research were fuchsine water, alcohol 95%, ethanol 96%, ginger in various varieties derived from the Research Institute of Medicinal and Aromatic Plants (Balitro) Bogor, West Java, Indonesia carbolic fuchsine, carbolic acid gentian violet, paper disc, clindamycin 300 mg, solution of $FeCl_3$ 1% HCl solution, a solution of H_2SO_4, solution of chloroform, Lugol, medium MHA (Mueller Hinton Agar), medium MHB (Mueller Hinton Broth), medium for biochemical test, methylene blue, NaCl physiological, reagents Lieberman Buchard, reagents Wagner and Mg powder.

Preparation of ginger extracts

Ginger rhizomes were washed in running water and afterward sliced finely. Once sliced, the rhizomes were dried and mashed into powder. A total of 300 grams of each powdered rhizome were extracted by maceration (soaking) using 1.5 L of ethanol 96% (1:5) for 3 x 24 hours. Every 24 hours, the result from each maceration was filtered. Afterward, all filtrates from the first, second and third days of maceration were combined and then evaporated using a rotary vacuum evaporator.

Phytochemical test of rhizome extract

Phytochemical test of ginger extract includes the examination of alkaloids, flavonoids, tannins, saponins, triterpenoids, steroids and phenol content (Harborne 1984).

Isolation and Identification of Bacteria from Acne

Acne samples were taken from two male and female patients. Each sample was placed into the bottle containing Mueller-Hinton Broth (MHB) medium. Furthermore, the medium was incubated for 24 hours at 37°C. MHB positive medium was characterized by the presence of turbidity, meaning there is the growth of microorganisms on it. A total of 1 mL MHB positive medium was placed in a petri dish, and 20 mL of Mueller Hinton Agar (MHA) medium was added to the petri dish. After, the medium was homogenized and incubated for 24 hours at 37°C. The growth of bacterial colonies was observed, this includes colony morphology (color, shape, edge, surface and elevation colonies), bacterial cell types (Gram and spores staining) and biochemical tests (fermentable carbohydrates, motility, Simon Citrate, Methyl Red and Voges-Proskauer test). Three isolated bacteria with different colony morphology were selected for further examination on their antibacterial activity.

Determination of Minimum Inhibitory Concentration (MIC)

MIC tests were carried out by using several sterile test tubes filled with 0.5 mL of medium MHB and 0.5 mL antibacterial test (ethanol extract of ginger), each with a set concentration of 80, 40, 20, 10, 5, 2,5, 1.25 and 0.625%. Afterward, the bacterial suspension was prepared that has been synchronized with the turbidity equals to 0.5 McFarland (1.5×10^8 CFU mL^{-1}). A total of 1 ose of bacterial suspension was placed into a test tube containing MHB and antibacterial test medium, then incubated for 24 hours at 37°C. The lowest concentration of antibacterial test in a test tube (indicated by cloudy appearance), expressed as the MIC.

Test on antibacterial activity of ethanol extracts of ginger against acne-origin bacterial isolates

The acne bacterial isolates were 24 hours incubated on agar slant. Furthermore, these isolates were taken using ose and placed into a test tube containing physiological saline. The turbidity was measured up to 0.5 McFarland (1.5×10^8 CFU mL^{-1}). Afterward, the isolates were placed into a sterile petri dish containing 1 mL of bacterial suspension, with the addition of 20 mL of medium MHA. A mixture of medium and the bacterial suspension was homogenized and then left to freeze.

In this study, antibacterial activity test was conducted using the method described in CLSI (1998). Briefly, paper discs were soaked in test tubes which contain a predetermined concentration of ginger extracts. The concentrations used for the extract of var. Emprit and the gajah ginger were 20, 17.5, 15, 12.5, 10 and 7.5%. Whereas for the red ginger the concentration of extract used were12.5, 10, 7.5, 5, 2.5 and 2%. As a comparison to an antibiotic, clindamycin 30 mg mL^{-1} was also prepared, then left for approximately 30 minutes. Afterward, paper discs were taken in a test tube and placed in a petri dish containing a mixture of 20 mL and 1 ml suspension origin of acne bacteria. The medium was then incubated at 37°C for 18-24 hours, before measurement on the diameter of formed clear zone around the paper disc using a ruler.

Data analysis

Statistical Software. Data obtained from the experiment was analyzed using two-way ANOVA, where the first factor was the acne bacterial isolates and the second was the concentration of extracts or the antibiotic. When the significance was observed, the Tukey test was performed with the credence of 95%.

RESULTS AND DISCUSSION

Ethanol extracts

In this study, extraction of the rhizomes of three different ginger varieties was conducted using maceration in 96% ethanol solvent for three days (3 x 24 h) cycles. The liquid extracts obtained were concentrated in a rotary vacuum evaporator to harvest the viscous extract. This extraction method obtained 88.89, 99.98 and 89.30 g of viscous extract from the initial mass of 1,810, 1,970 and 1,910 g of emprit, gajah and red variants dried-rhizome ginger, respectively. Additionally, the phytochemical test revealed that the secondary metabolites were found in all three types of ginger extract (Table 1).

Isolation and identification of acne-origin bacteria

The isolation of bacteria from acne revealed three bacterial colonies with various colors. Apparently, the colonies morphology of the isolated bacteria had different characteristics in spite of they shared a spherical shape. Yellowish-white bacteria (isolate 1) have convex elevation colonies with flat edges, smooth and shiny surfaces. The white color bacteria (isolate 2) has a flat elevation and ledges colonies with a smooth surface. The bacteria with bone white color (isolate 3) has convex elevation colonies with serrated edge, smooth and shiny surface. These three isolates bacteria were obtained and identified. Based on the identification, all isolates were categorized as Gram Positive bacteria that are catalase-positive bacteria and they do not produce spores. Bacterial isolates 1 and 3 have the ability to ferment sugars (glucose, lactose, maltose, mannitol and saccharose), but the fermentation process does not produce gas. Negative results were obtained from the Methyl Red and Voges-Proskauer tests. In contrast, the Simmons Citrate test revealed a positive result, it means that the bacteria are able to use citrate as a carbon source. The motility test demonstrated that the bacterial isolates 1 and 3 were classified as motile and were expected to have a flagellum. In contrast to the results obtained in these isolates, bacterial isolates 2 were not able to ferment all sugars. These isolates were only able to ferment glucose, maltose, mannitol and saccharose. In MR test, all isolates revealed positive results, whereas negative results appeared in VP test. These bacteria can use citrate as a carbon source, which supported by positive results in SC test. The motility test showed that the bacterium was classified as non-motile flagella that have not been anticipated. The identified bacterial isolates were then compared with the information in the Bergey's Manual of Determinative Bacteriology (Holt and Krieg 1994) book. According to the analysis of comparison, the isolates 1 and 3 belonged to the genus *Propionibacterium*, while isolates 2 belongs to the genus *Staphylococcus*.

Determination of Minimum Inhibitory Concentration (MIC)

MIC was defined as the lowest concentration of ginger ethanolic extract that can inhibit the growth of acne-origin bacteria. In determining the MIC values with liquid dilution, the turbidity formed upon the medium is an indicator of bacterial growth. The obtained MIC for the three ginger ethanolic extract against the acne-isolated bacteria is presented in Table 2.

Table 1. Results of phytochemical test ethanol extract of ginger

Compound type	Ginger extract			Indicator of positive reaction
	Var. emprit	Var. gajah	Var. red	
Alkaloid	-	-	-	Presence of orange deposits
Flavonoid	+++	++	+++	Yellow colored
Tanin	-	-	+	Green-/redish colored
Saponin	+	+	+	Presence of foam
Triterpenoid/ steroid	+	+	+	The presence of green, red, blue or brown ring
Phenol	++	++	+++	The presence of black color

Note: (-): absence; (+): presence; number of (+) indicates the relative color intensity

Table 2. MIC for the three ginger ethanolic extract against the acne-isolated bacteria

Ginger extract	Bacterial isolates	Concentration of extract (%)							
		80	40	20	10	5	2.5	1. 25	0.625
Emprit ginger	*Propionibacterium* sp.1	-	-	-	-	+	+	+	+
	Staphylococcus sp.	-	-	-	-	-	+	+	+
	Propionibacterium sp.2	-	-	-	+	+	+	+	+
Common ginger	*Propionibacterium* sp.1	-	-	-	-	+	+	+	+
	Staphylococcus sp.	-	-	-	-	+	+	+	+
	Propionibacterium sp.2	-	-	-	+	+	+	+	+
Red ginger	*Propionibacterium* sp.1	-	-	-	-	-	-	+	+
	Staphylococcus sp.	-	-	-	-	-	+	+	+
	Propionibacterium sp.2	-	-	-	-	-	+	+	+

Note: (+) contained bacterial growth, (-) no bacterial growth

Table 3. Antibacterial activity of the three ethanolic-ginger extract against the isolated acne-bacteria

Antibacterial test (B)	Bacteria tested (A)		
	***Propionibacterium* sp.1**	***Staphylococcus* sp.**	***Propionibacterium* sp.2**
	Clear zone diameter (mm)		
Emprit (%)			
7.5	5.5 ^{ab} EF	6.0 ^{a} H	1.0 ^{c} G
10	7.0 ^{a} DE	6.5 ^{a} GH	2.5 ^{b} FG
12.5	8.5 ^{bcd} CDE	9.5 ^{abc} EFG	6.5 ^{cd} E
15	8.5 ^{cd} CDE	12.0 ^{ab} DE	10.5 ^{bc} D
17.5	11.5 ^{bc} BC	14.5 ^{ab} CD	14.5 ^{ab} BC
20	12.5 ^{b} B	17.0 ^{a} BC	17.5 ^{a} AB
Gajah (%)			
7.5	5.5 ^{bc} GH	15.0 ^{a} EF	4.5 ^{c} F
10	6.5 ^{bc} EFGH	15.5 ^{a} DEF	5.0 ^{c} EF
12.5	8.0 ^{bc} EFG	18.0 ^{a} CDE	6.0 ^{cd} DEF
15	9.5 ^{bc} DE	19.5 ^{a} C	7.5 ^{cd} CDEF
17.5	12.5 ^{b} CD	23.0 ^{a} B	9.0 ^{c} CD
20	16.0 ^{b} B	28.0 ^{a} A	9.5 ^{c} BC
Red (%)			
2	6.0 ^{b} G	9.5 ^{a} G	1.0 ^{c} G
2.5	7.0 ^{b} FG	13.0 ^{a} F	1.5 ^{c} G
5	7.5 ^{bc} EFG	16.5 ^{a} DE	5.5 ^{c} F
7.5	10.0 ^{c} DE	18.0 ^{a} D	15.0 ^{b} CDE
10	11.5 ^{c} CD	21.0 ^{a} C	16.0 ^{b} BCD
12.5	13.5 ^{c} BC	25.0 ^{a} B	17.5 ^{b} BC
Clindamycin (mg mL^{-1})			
30	16.5 ^{cd} A	29.5 ^{a} A	18.5 ^{bc} AB

Note: Mean followed by similar small- and capital letters to the row- and column-direction, respectively, shows no significant difference according to Tukey test with 95% of confidence level ($\alpha = 0.05$)

The three varieties of ginger had different MIC values (Table 3). Emprit Ginger had MIC at concentration 10 and 5% against *Propionibacterium* sp.1 and *Staphylococcus* sp, respectively. On the other hand, the MIC of the gajah ginger against *Propionibacterium* sp.1 and *Staphylococcus* sp. were at concentration 10 and 20%, respectively. Additionally, the red ginger had MIC against *Propionibacterium* sp.1 at concentration 2.5%, whereas at 5% for *Staphylococcus* sp. and *Propionibacterium* sp. 2.

The difference results in MIC values of all three varieties of ginger showed that they possessed different in ability in inhibiting the growth of acne-origin bacteria. The occurance due to the differences in active substances from each of the ginger extract. As shown in phytochemical test results (Table 1), for instance, tannin was uniquely found in red ginger instead of the other 2 varieties. It was known that ginger var. red contains tannin that is not owned by the other two varieties, so hence the formation of bacterial cell

wall tests was disrupted In addition, the content of flavonoids and phenolic possessed by ginger var. red is higher than in other vareities. Therefore, the process denaturation of protein test of bacterial cells become faster and may lead the bacteria death. Thus, this is one of the reasons why MIC value of red ginger wasThese are one of the causes of ginger var. red has MIC value is smaller lower than variety of gajah and emprit.

Antibacterial activity of rhizome extracts

Antibacterial activity test of rhizome extracts of all the three ginger varieties showed the formation of a clear zone around the paper disc. The presence of the clear zone indicated that substances in the extract could inhibit the growth of acne-origin bacteria.

Based on the MIC values as shown in Table 2, the antibacterial activity of rhizome extract of ginger var. emprit was applied using 7.5, 10, 12.5, 15, 17.5% serial concentration.

Discussion

Ginger (*Zingiber officinale*) has long been used as naturopathy due to their potential antimicrobial activity properties against different microbial pathogens. verall, this study shows that the ethanolic extract from the three ginger varieties (emprit, gajah, and red) have different abilities to inhibit the growth of acne-origin bacteria where the highest capability was observed on the red ginger. This result is confirmed by the study from Masniari (2011) who found that the red ginger was effective in controlling *Staphylococcus aureus, Staphylococcus epidermidis* and *Streptococcus agalactiae,* which responsible for mastitis. However, the antimicrobial properties from red ginger were still lower than the clindamycin 30 mg mL^{-1} antibiotics.

The differences in the capabilities of all three ginger varieties were probably caused by their bioactive compounds which act as antibacterial. In addition, the concentration used also affects the antibacterial effect, considering it can be hampering the growth of bacteria as well as killing the bacteria directly. Most of the ginger's extracts show high antibacterial activity against both gram-positive and gram-negative bacteria. Therefore, ginger can provide protection to a certain extent against bacterial pathogens (Hindi et al. 2014).

Among existed factors, the reaction between the active ingredient with a medium, incubation temperature, acidity, media components, antibacterial stability, inoculum size, incubation time and the metabolic activity of bacteria are considered as the important factors in affecting the antibacterial activity (Zu et al. 2012). Biological capabilities of each bacterium also vary in response to antibacterial properties and one of the most influential factors were the differences in the structure of bacteria cell walls, such as peptidoglycan thickness. This parameter determines the sensitivity of bacteria to an antibacterial agent because the peptidoglycan found in the cell walls of bacteria was sensitive to attack by bacteria. Ginger extracts were obtained using solvents, n-hexane, ethyl acetate, ethanolic soxhlet, and water. The extracts were assayed for antibacterial activity and bacterial growth inhibition activity. Except the water extract, the results showed that all the extracts have antibacterial activity and generally, inhibition of bacterial growth was dose dependent. The results also showed that ginger extracts possesses antibacterial properties and could be used for the treatment of bacterial infections (Malu et al. 2009).

In conclusions, the ethanolic extract of emprit, gajah and red ginger varieties have different abilities to inhibit the growth of acne-origin bacteria. Ginger has a value of MIC against *Propionibacterium* sp.1, *Staphylococcus* sp. and to *Propionibacterium* sp.2 as much as 10, 5 and 20%, respectively. The red ginger has MIC value against *Propionibacterium* sp.1 as much as 2.5%, as well as *Staphylococcus* spp. and *Propionibacterium* sp.2 by 5%. The test on antibacterial activity demonstrated that the highest antibacterial activity was obtained from the ethanolic extract of red ginger with concentration of 12.5% producing a clear zone of 13.5, 25.0 and 17.5 mm against *Propionibacterium* sp.1, *Staphylococcus* sp. and *Propionibacterium* sp.2, respectively.

REFERENCES

Derrida M. 1999. Common spices protect bacteria during irradiation 1999. Am Chem Soc 2: 270-275.

Harborne JB. 1984. Phytochemical Methods A Guide to Modern Techniques of Plant Analysis, 2nd ed., Chapman and Hall, London.

Hindi NKK, Al-Mahdi ZKA, Chabuck ZAG. Antibacterial activity of the aquatic extract of fresh, dry powder ginger, apple vinegar, extract of fresh ginger and crud oil of ginger (*Zingiber officinale*) against different types of bacteria in Hilla City, Iraq. Intl J Phama Pharmaceut Sci 6 (5): 414-417.

Holt JG, Krieg NR (eds.). 1994. Bergey's Manual of Determinative Bacteriology. 9th ed. The Williams & Wilkins Co., Baltimore.

Joe MM, Jayachitra J, Vijayapriya M. 2009. Antimicrobial activity of some common spices against certain human pathogens, J Med Pl Res 3 (11): 1134-1136.

Kumesan YAN, Paulina VY, Yamlean, Hamida SS. 2013. The gel formulation and test activities anti-acne narcissus bulb extract (*Crinum asiaticum* L.) Against *Staphylococcus* aureus secara in vitro. Sci J Pharmaceut UNSTRAT 2 (2): 18-26.

Malu SP, Obochi GO, Tawo EN, Nyong BE. 2009. Antibacterial activity and medicinal properties of ginger (*Zingiber officinale*). Glob J Pure Appl Sci 15 (3): 365-368.

Masniari P. 2011 The effect of red ginger (*Zingiber officinale* Roscoe) extract on the growth of mastitis causing bacterial isolates. African J Microbiol Res 5 (4): 382-389.

Movita, T. 2013. Acne vulgaris. CDK-203 40 (4): 269-272.

Setyawan AD. 2002. Variation on ginger (*Zingiber officinale* Rosc.) varieties based on chemical constituent of volatile oils. BioSMART 4 (2): 48-54. [Indonesian]

Surh YJ, Park KK, Chun KS, Lee LJ, Lee E, Lee SS. 1999. Anti-tumor-promoting activities of selected pungent phenolic substances present in ginger. J Environ Pathol Toxicol Oncol 18 (2): 131-139.

Tanghetti EA. 2013. The role of inflammation in the pathology of acne. Aesthet J Clin Dermatol 6 (9): 27-35.

Zu YG, Yu HM, Liang L, Fu YJ, Efferth T, Liu X, Wu Nan. 2010. Activities of ten essential oils towards *Propionibacterium acnes* and PC-3, A-549 and MCF-7 cancer cells. Molecules 15 (5): 3200-3210.