Indonesian Journal of Chemical Research

http://ojs3.unpatti.ac.id/index.php/ijcr Indo. J. Chem. Res., 8(2), 180-185, 2021

Concentration Effect of Leaf Extract from Kekara Laut (*Canavalia Maritima* Thou.) in inhibiting of *Staphylococcus Epidermidis* Bacteria with a Statistical Science Approach

Irman Idrus[1], Fajar Kurniawan[1], Faizal Mustapa[2], Dwiprayogo Wibowo[3]*

[1]Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Pelita Ibu Kendari, Anduonohu, Poasia, Kendari 93231, Southeast Sulawesi, Indonesia

[2]Department of Aquaqulture, Faculty of Sciences and Technology, Institut Teknologi dan Kesehatan Avicenna, Lepo-Lepo, Kendari 93117 – Southeast Sulawesi, Indonesia

[3]Department of Environmental Engineering, Faculty of Engineering, Universitas Muhammadiyah Kendari, Kendari 93117 - Southeast Sulawesi, Indonesia.

**Corresponding Author: dwiprayogowibowo@yahoo.com*

Received: September 2020
Received in revised: November 2020
Accepted: December 2020
Available online: January 2021

Abstract

Importantly to study the coastal vegetation from Kekara Laut (Canavalia maritima Thou.) to observe the antibacterial agents towards *Staphylococcus epidermidis*. The purpose of this study to examine the variation concentration effect of an ethanol leaf extract from *Canavalia maritima* Thou. in inhibiting the growth of *S. epiderimidis* bacteria. The leaf of *Canavalia maritima* Thou. was prepared using a physicochemical method to obtain ethanol extract, then varied in several concentrations, namely 5% w/v; 10% w/v; 15% w/v; and 20% w/v. Based on these results, we obtain the ethanol leaf extract can inhibit the growth of *S. epidermidis* with a concentration of 15% w/v for 24 hours having an inhibition zone of 17.17 mm. The statistical analysis test was very significantly different from all antibacterial tests, the value of F count was 682.1 with F table (3.48), at the level of $\alpha = 0.05$ (3.48) and $\alpha = 0.01$ (5.99), which indicates that H_0 is rejected, but using the Variance method in the Newman-Keuls range approach shows that each concentration has a good inhibitory ability. The use of *Canavalia maritima* Thou. leaf extract in low concentrations can significantly inhibit bacterial growth (bacteriostatic).

Keywords: Ethanol, extract, leaf, Canavalia maritima Thou., *antibacterial*

INTRODUCTION

The last few years the exploration of active compounds as an alternative of natural medicine from plants and organisms were intensified by the researchers under organic chemistry and pharmaceuticals to obtain the information related to its potential activities as antimicrobial materials (Ahmad, A., 2016; Rahmawati and Sudjarwo, 2011). This condition due to the several chemical compounds have different benefits to inhibit several diseases. Today, the modern medical also began to re-study the utilization of traditional drugs as it shows no highly significant impact on the emergence of side effects than synthetic drugs (Subroto and Harmanto, 2013).

Traditional medicine is considered safer compared with synthetic drugs because it can literally be neutralized in the human body, increase the antibody against virus/ bacterial, and safe to be consumed (Astarina et al., 2013; Kiromah et al., 2019). However, the utilization of various plants as medicine material is required intensive study related invitro and invivo tests to obtain their activity to various diseases (Ariani et al., 2017; Pranoto et al., 2012). One of the diseases that suffered by the several community is the infection caused by bacteria (Ariani and Riski, 2018; Kursia et al., 2016). Bacteria is an microorganisms and only identified by using microscope tools (Bin dan Vitria., 2018).

Besides that, pathogenic bacteria are more dangerous and can lead to infection both sporadic and endemic as in the *Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* (Gaol et al., 2017; Taslim and Maskoen, 2016). In addition, importantly to investigate bioactive compounds material from nature should be explorated to decrease bacterial resistance of pathogens as antibiotics and dependency of synthetic drugs against human body (Kurniawati et al., 2016; Mukti, 2017).

The exploration of biodiversity within the scope of the beach vegetation is very unique as well as to benefit the coast community to be able to take

advantage of herbs the infection diseases (Bhagya and Sridhar, 2009; Salimi et al., 2019). This is certainly very beneficial to the Indonesian population because most of the majority of living partially coated.

One of the beach vegetation that often encountered in Indonesia is the *Canavalia maritima* Thou. which has not been considered intensively by some researchers related to antibacterial potential of *S. epidermidis*. Several previous studies have reported that marine cabling contains the potentially integrated antibacterial and antiparaciteic compounds because it can inhibit the cell adsion and thin layers of cells by interrupting cell signal (Arrudapat et al., 2013). This compound is a derivative of proteins that function to bind gloprotein body cells and glycolipids expressed on the surface of the cell, this condition can disease the surface of the bacterial cell wall (Iordache et al., 2015). The potential as an antibacterial of *Canavalia maritima* Thou. can be tested in some bacteria. Therefore in this study aims to test of ethanol extract from *Canavalia maritima* Thou. leaf to obtain potential as an antibacterial material against *S. Epidermidis* with a statistical science approach to determine the effectiveness as antibaterial.

METHODOLOGY

Apparatus and Materials

In this research are a set of glassware (IWAKI-PYREX), Rotary Evaporator (BUCHI), spray bottles, blender, autoclave, laminar, oven, term, analytic scales. While the material used is distilled water, ethanol 70% w/v (technical), pure briber *S. epidermidis*, *Canavalia maritima* Thou. leaf, disc paper, nutrient medium for NaCl 0.9% w/v, *Muller Hilton Agar* (MHA), Na.CMC 1% w/v, paper label, sterile swab, and aluminium foil.

Experimental

Preparation and Extraction

The initial stage in this research is the sample preparation through the physical stage, where the *Canavalia maritima* Thou. leaf collected from the middle-sulawesi then is cleaned from the impurities. The leaf is washed using water flowing to clean, droign, and dried under the sunlight exposure to dry the sample.

Furthermore, the sample was milled by using blender and sifted to smoothness of 80 mesh. Sample was weighed 500 grams, then incorporated into the maceration tank and incorporated ethanol solvent 70%. Maceration was conducted for 5×24 hours with occasional shipments shuffled to increase contact between solvents with a sibling sample. Then filtered using filter cloth to separate between residues and rough extracts. The last stage is separated rough extract by using a rotary evaporator until the pure extract is obtained.

Antibacterial test against *S. epidermidis*

The entire tools used for antibacterial testing was sterilized using detergent, distilled water, ethanol and included in the autoclave at 121°C with 1 atm for 15 minutes. In addition, the sample solution was used by varying concentration extract such as 5% w/v; 10% w/v; 15% w/v; and 20% w/v by weighing 5 g; 10 g; 15 g and 20 g extracts of sample and also negative control of 100 mL of Na.CMC solution 1% w/v.

To improve the quality of antibacterial testing, the testing rejuvenation of the test of the NA Media incubated for 1×24 hours. Furthermore, testing to the test bacteria prepared MHA medium that has sold inserted each Paperdisc that has been soaked in the variations of the ethanol extract of *Canavalia maritima* Thou. leaf. The negative control used is Na.CMC 1% w/v. Then put on the surface media that has been complaced aseptically by using a sterile tweezers, with a distance of 2-3 cm from the edge of the Petri cared, then incubated at 37°C for 1×24 hours.

RESULTS AND DISCUSSION

Antibacterial Test against *S. epidermidis*

This study uses ethanol extract of *Canavalia maritima* Thou. leaf with the variation concentration of 5% w/v, 10% w/v, 15% w/v, and 20% w/v. The *Canavalia maritima* Thou. leaf has extracted using the maceration method (immersion) in order to the texture of sample is soft which is require the extraction method as coolly easily and quickly. Universally, this maceration technique has been widely applied in the exploration of secondary metabolite compounds of plants because it is considered excellent in attracting some of the components of active compounds in plants.

Importanlty, in this process is the sample size used should be smaller when contacting with the solvent in order to get optimal extraction because the high surface area of the leaf contact is the greater (Sa'adah and Nurhasnawati, 2017). In addition, the volume of solvent used should be more added because the capability of the saturation also plays a role in its ability to extract. If the saturation conditions, the ability of the extraction is getting weaker (Triastiari and Harijono, 2019). Ethanol solvent for maceration is very safe for antibacterial testing when compared to methanol because of the nature of the polarity and electronegative of the ethanol is weaker than methanol due to the distribution of electrons from the CH_3-CH_2-

groups providing stability on the –OH groups (Surtina et al., 2020).

Furthermore, the rough extract is separated using rotary evaporator to obtain a concentrated extract from *Canavalia maritima* Thou. leaf. The solvent is evaporated under ambient temperature to obtain dry extract. In this study the test bacteria was applied in this study using *S. Epidermidis* is a pathogenic bacteria in humans.

Table 1. The results of diameter zone by using *Canavalia maritima* Thou. leaf extract against *S. epidermidis*

Bacterial test	Repeatability	Diameter zone (mm)					Total
		5% w/v	10% w/v	15% w/v	20% w/v	Na.CMC 1% w/v Control (-)	
Staphylococcus epidermidis	1	13.00	14.00	16.50	18.50	0	62.00
	2	14.00	15.00	17.50	19.50	0	66.00
	3	13.50	15.00	17.50	19.50	0	65.50
Total		40.50	44.00	51.50	57.50	0	193.50
Average		13.50	14.67	17.17	19.17	0	
STD		0.50	0.57	0.57	0.57	0	

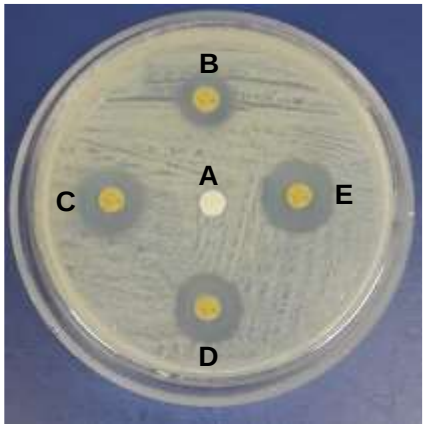

Figure 1. Antibacterial activity test results of *Canavalia maritima* Thou. leaf extract against *S. Epidermidis*, A. Na.CMC 1%w/v; B. 5 %w/v; C. 10%w/v; D. 15%w/v; E. 20%w/v.

The inhibition test was conducted by using the pour method. The MHA medium is persecuted persecutively into the Petri's cup is allowed the bacterial suspension tempered at aseptic above the MHA medium surface then laid the disc paper that previously immersed with samples and controls then placed in a clockwise. The result of antibacterial test of *S. Epidermidis* can be seen in Figure 1 and Table 1.

Based on the results in Figure 1 and Table 1 are known that the ethanol leaf extract with a variation of concentration of 5% w/v, 10% w/v, 15% w/v, and 20% w/v can inhibit the growth of *S. Epidermidis* with the greatest concentration of the inhibit zone is shown at concentration of 20% w/v. However, the closure of the diameter zone of the 20% w/v is relevanted with 15% w/v. According to Muharammy et al. (2016) that the ability inhibitory zone of the compound as an antibacterial is shown that the 15-20 mm range is categorized in moderate hamps. While the inhibitory diameter zone more than 20 mm shows the power of stronger. In this study, the ethanol extract at a concentration of 15% w/v and 20% w/v are categorized as moderate hamps as an antibacterial.

According to Collins, (2018) the *Canavalia maritima* Thou. has a characteristic of bacteriostatic is a condition caused by antibacterial compound inhibits the growth / development of bacteria remains, so that the ability of impartial power may be decreased. Other factors may also affect such levels of concentration in ethanol because the difference in compound content is bound to each concentration, where the higher concentration of extract used was proportional with the antibacterial compounds contained in the extract. The result of the diameter zone showed the difference in each concentration due to differences in the concentration of active substances.

Increased concentrations will generally be followed by an increase inhibition diameter zone as construction by Kusuma et al. (2017) that concentration and chemicals will affect microorganisms where the highest concentration will cause the death of microorganisms. However, the inhibition zone formed does not always follow this rule, because several factors may affect the results of the inhibitory power such as ability and the rate of diffusion of active ingredients in the medium, like the growth rate of microorganisms, sensitivity of microorganisms against active substances, and the thickness and viscosity of the medium (bin and Vitria, 2018).

The antibacterial chemical compounds contained in the marine of the sackeline of lectin and alkaloid compounds working by disrupting the components of

Table 2. Statistical Analysis results by finger variety

Source of uniformity	DB	JK	KT	F_{count}	F_{Table}	
					0.05	0.01
Treatment	4	682.1	170.525			
Mistake	10	2.5	0.250	682.1**	3.48	5.99
Total	14					

Table 3. The average treatment by arrange from smallest to the largest data

	A	B	C	D	E
Treatment	Na.CMC 1%w/v Control (-)	Extract 5% w/v	Extract 10% w/v	Extract 15% w/v	Extract 20% w/v
Average	0	13.50	14.67	17.17	19.17

peptidoglican in bacterial cells, as the cell walls are not formed intact and cause deaths to the bacterial cell (Nurdin et al., 2018).

Statistical Analysis Test

The results of statistical analysis using the complete randomized design (CRD) by using SPSS application show that the distilled water as a negative control, the *Canavalia maritima* Thou. leaf has significantly diameter zone to inhibit *S. Epidermidis* where the inhibition zone as antibacterial shows that the F_{count} value of 682.1 is greater than the F_{table} at the 0.05 (3.48) and F_{table} at the 0.01 (5.99) (Table 2). Based on the CRD method in Table 2 shows that the ethanol extract from *Canavalia maritima* Thou. can inhibiting of the *S. Epidermidis*. Although the results of the finger variety (Table 2) is the H_0 denied F_{Count} 3.48 (F_{Table} 0.05), we can observe the effect of each treatment using the variance test in the Newman-Keuls range approach (Table 3). The last step of generating comparative between treatments:

A opponent B = 13.50 > 1.38 (significant)
A opponent C = 14.67 > 1.70 (significant)
A opponent D = 17.17 > 1.90 (significant)
A opposite E = 19.17 > 2.05 (significant)
B opposite C = 1.17 > 1.38 (significant)
B opposite D = 3.67 > 1.70 (significant)
B opponent E = 4.20 > 1.90 (significant)
C opponent E = 4.07 > 1.70 (significant)
D opponent E = 2.0 > 1.38 (significant)
C opponent d = 2.5> 1.38 (significant)

Based on CRD method and analysis of variance can be concluded that the variation of concentration of *Canavalia maritima* Thou. leaf extract in the low concentration as significantly to inhibit *S. Epidermidis*.

CONCLUSIONS

Based on the results obtained indicate that the *Canavalia maritima* Thou. leaf extract can inhibit the growth of *S. Epidermidis* bacteria with a concentration of 15% w/v incubated during 1×24 hours having a 17.17 mm inhibitory zone (medium). Statistical analysis test was very significant or different to the entire antibacterial testing obtained the value of F_{Count} 682.1 with F_{table} (3.48), at the level of $\alpha = 0.05$ (3.48) and $\alpha = 0.01$ (5.99) which showed H_0 was rejected but using variance method in the Newman-Keuls range approach showed that each concentration had good inhibition power capabilities. The use of *Canavalia maritima* Thou. leaf extract in low concentrations can significantly inhibit bacterial growth (bacteriostatic).

ACKNOWLEDGMENT

We acknowledge for Laboratorium Phytochemistry and Microbiology at Faculty of Farmacy, Universitas Indonesia Timur and Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Pelita Ibu Kendari to conduct this research.

REFERENCES

Ahmad, A., and Firdaus, S., 2016. Potensi Ekstrak Metanol Kulit Batang Lannea coromandelica (Houtt.) Merr. Terhadap Staphylococcus aureus dan Analisis Metabolit Sekunder Utamanya, *Indo. J. Chem. Res.*, 4(1), 362–366.

Ariani, N., and Riski, A., 2018. Aktivitas Ekstrak Etanol Kulit Buah Pisang Kepok Mentah (Musa paradisiaca forma typica) Terhadap Pertumbuhan *Candida albicans* Secara In Vitro, *J. Pharmascience*, 5(1), 39–44.

Ariani, N. K. I. R., and Kurniadewi, F., 2017. Uji Aktivitas Inhibisi Enzim ±-Glukosidase secara In Vitro dari Ekstrak Metanol Daun Cryptocarya

densiflora Blume dan Fraksi-Fraksinya, *J. Ris. Sains Dan Kim. Terap.*, 7(1), 14–20.

Arrudapat C. T. T., Carneiro, V. A., Neves, C. C., Sousa Duarte, H. de, Queiroz Martins, M. G. de, Sousa Arruda, F. V., Vasconcelos, M. A. de, dos Santos, H. S., Silva Cunha, R. M. da, Cavada, B. S., Teixeira, E. H., 2013. A ConA-like Lectin Isolated From *Canavalia Maritima* Seeds Alters The Expression Of Genes Related To Virulence And Biofilm Formation in *Streptococcus mutans*, *Adv. Biosci. Biotechnol*, 04(12), 1073–7078.

Astarina, N. W. G., Astuti, K. W., Warditiani, N. K., 2013. Skrining Fitokimia Ekstrak Metanol Rimpang Bangle (Zingiber purpureum Roxb.), *J. Farm. Udayana*, 2(4), 279691.

Bhagya, B., and Sridhar, K. R., 2009. Ethnobiology of Coastal Sand Dune Legumes of Southwest Coast of India, *Indian J. Tradit. Knowl*, 8(4), 611–620.

Bin, A. K. M., and Vitria, L., 2018. Uji Aktivitas Ekstrak Etanol Buah Kecombrang (Nicolai speciosa) Terhadap *Staphylococcus aureus* Dan Pseudomonas aeruginosa, *Java Heal. Jounal*, 5(1), 1–8.

Collins, E., 2018. *Antibacterial: Synthesis, Properties and Biological Activity. In Protests and Riots Past: Present and future perspectives*, Nova Science Publishers, Inc., USA.

Gaol, Y. E. L., Erly, E., Sy, E., 2017. Pola Resistensi Bakteri Aerob pada Ulkus Diabetik Terhadap Beberapa Antibiotika di Laboratorium Mikrobiologi RSUP Dr. M. Djamil Padang Tahun 2011-2013, *J. Kesehat. Andalas*, 6(1), 164–170.

Iordache, F., Ionita, M., Mitrea, L. I., Fafaneata, C., Pop, A., 2015. Antimicrobial and Antiparasitic Activity of Lectins, *Curr. Pharm. Biotechnol*, 16(2), 152–161.

Kiromah, N. Z. W., Widiastuti, T. C., Krisdiyanti, Y., Kurniawan, Y., 2019. Tingkat Penggunaan dan Kesadaran Masyarakat dalam Konsumsi Obat Tradisional Di Wilayah Kerja Puskesmas Gombong, *J. Ilmu Kesehatan Keperawatan*, 15(1), 47–53.

Kurniawati, A., Lukman, D. W., Wibawan, I. W. T., 2016. Resistensi Antibiotik pada Salmonella Isolat Sapi Bakalan Asal Australia yang Diimpor Melalui Pelabuhan Tanjung Priok Jakarta, *J. Vet. Sept.*, 17(3), 449–456.

Kursia, S., Lebang, J. S., Nursamsiar, N., 2016. Uji Aktivitas Antibakteri Ekstrak Etilasetat Daun Sirih Hijau (Piper betle L.) terhadap Bakteri Staphylococcus epidermidis, *Indones. J. Pharm. Sci. Technol.*, 3(2), 72–77.

Kusuma, M. S., Susilorini, T. E., Surjowardojo, P., 2017. Pengaruh Lama Dan Suhu Penyimpanan Ekstrak Daun Sirih Hijau (Piper Betle Linn) Dengan Aquades Terhadap Daya Hambat Bakteri Streptococcus Agalactiae Penyebab Mastitis Pada Sapi Perah, *Ternak Trop. J. Trop. Anim. Prod*, 18(2), 14–21.

Muharammy, F., Machmud, R., Nelis, S., 2016. Perbedaan Daya Hambat Obat Anestesi Lokal Lidocaine 2% dan Articaine 4% terhadap Pertumbuhan Bakteri Porphyromonas gingivalis Secara In Vitro, *Andalas Dent. J.*, 4(2), 89–97.

Mukti, A., 2017. The Antibiotic Resitance Escherichia Coli In Broiler Meat at Rukoh Market, *J. Ilm. Mhs. Vet.*, 1(3), 492–498.

Nurdin, M., Yanti, N. A., Suciani, Watoni, A. H., Maulidiyah, Aladin, A., Wibowo, D., 2018. Efficiency of Ilmenite Photocatalyst Material as Modelling for Antimicrobial Activity, *Asian J. Chem.*, 30(6), 1590–1592.

Pranoto, E. N., Ma'ruf, W. F., Pringgenies, D., 2012. Kajian Aktivitas Bioaktif Ekstrak Teripang Pasir (Holothuria Scabra) Terhadap Jamur *Candida Albicans*, *J. Pengolah. Dan Bioteknol. Hasil Perikanan*, 1(2), 1–8.

Rahmawati, N., and Sudjarwo, E., 2011. Uji Aktivitas Antibakteri Ekstrak Herbal Terhadap Bakteri Escherichia Coli, *Ilmu-Ilmu Peternak.*, 24(3), 24–31.

Sa'adah, H., and Nurhasnawati, H., 2017. Perbandingan Pelarut Etanol Dan Air Pada Pembuatan Ekstrak Umbi Bawang Tiwai (Eleutherine americana Merr) Menggunakan Metode Maserasi, *J. Ilmu Manuntung*, 1(2), 149–153.

Salimi, Y., Bialangi, N., Abdulkadir, W., Parulian, B. R., 2019. Senyawa Triterpenoid dari Ekstrak N-heksana Daun Kelor (Moringa oleifera Lamk.) dan Uji Aktivitas Antibakteri Terhadap Staphylococcus aureus dan Escherichia coli, *Indo. J. Chem. Res.*, 7(1), 32–40.

Subroto, A., and Harmanto, N., 2013. *Pilih Jamu Dan Herbal Tanpa Efek Samping*, Elex Media Komputindo, Jakarta

Surtina, S., Sari, R. P., Zulita, Z., Rani, R., Roanisca, O., Mahardika, R. G., 2020. Potensi Antibakteri Ekstrak Daging Buah Kelubi (Eleiodoxa conferta) Bangka Belitung Menggunakan Microwave-Assisted Extraction (MAE), *Indo. J. Chem. Res.*, 7(2), 177–182.

Taslim, E., and Maskoen, T. T., 2016. Pola Kuman Terbanyak Sebagai Agen Penyebab Infeksi Di Intensive Care Unit Pada Beberapa Rumah Sakit Di Indonesia, *Anesth. Crit. Care*, 34(1), 56–62.

Triastiari, A., and Harijono, H., 2019. Pengaruh Pengeringan dan Lama Maserasi dengan Pelarut Ganda Etanol dan Heksana Terhadap Senyawa Bioaktif Kulit Buah Palem Putri (Veitchia merillii), *J. Pangan Dan Agroindustri*, 7(1), 18–29.