The antibacterial activity of Tembelekan leaf (*Lantana camara* L.) and Kopasanda leaf (*Chromolaena odorata* L.) extracts against *Staphylococcus aureus*

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Abstract

Staphylococcus aureus (*S. aureus*) is a round-shaped Gram-positive bacteria that caused infection in the human body. *S. aureus* infection can be inhibited by the use of antibiotics. However, these bacteria displayed resistance to some antibiotics. The purpose of this study is to determine the effects of Tembelekan (*Lantana camara L.*) and Kopasanda (*Chromolaena odorata L.*) leaf extracts against *S. aureus*. The method used in this study is the disc method to determine the inhibition zone. The test results showed there was no significant difference in the sensitivity of Tembelekan leaf extract (*L. camara L.*) and Kopasanda leaf extract (*C. odorata L.*) against *S. aureus* (P0.8>α0.05). However, based on the average value of the inhibition zone, Kopasanda leaf extract was showed higher inhibition zone compared to Tembelekan leaf extract. In conclusion, Tembelekan leaf and Kopasanda leaf at a concentration of 100% were produced inhibition zones of 0.6 cm and 0.8 cm respectively against *S. aureus.*

Introduction

Bacterial infections are invasion and multiplication of microorganisms (bacteria) in the body's tissues that produce signs and symptoms of an immune response. Bacteria can cause diseases in humans when they enter the body through the skin, nose, eyes, vagina or mouth. One of the bacteria that can cause infection in the human body is *Staphylococcus aureus* (*S. aureus*). *S. aureus* bacteria can cause purulent infections and abscesses that commonly affect children, the elderly and people whose immune system decreases.[1]

S. aureus infection can be inhibited by the use of antibiotics. Antibiotics are group of molecules, both natural and synthetic, which have the effect of suppressing or stopping a biochemical process in an organism, especially in the process of infection by bacteria. However, some bacteria have displayed resistance to certain types of antibiotics.[2]

According to the UPTD Health Laboratory Center (2012-2014), the pattern of resistance of *S. aureus* bacteria to 17 antibiotics from 2012 to 2014 was not the same or varied. In 2012 *S. aureus* was 6.9% resistant to antibiotics Amikasin, in 2013 it rose to 21.1%, and in 2014 it rose again to 26.1%. In 2012 *S. aureus* was 60% resistant to Ampicilin antibiotics, in 2013 it dropped to 52.6%, and in 2014 it rose again to 82.6%. *S. aureus* was 20% resistant to Amoxicillin antibiotics in 2012, in 2013 it rose 28.6%, and in 2014 it rose again to 77.8%. Likewise for other antibiotics, the resistance of *S. aureus* to different antibiotics will also be different.

Several studies reported of substances derived from natural ingredients that can inhibit the growth of bacteria, and which can be tested by using sensitivity testing techniques. Bacterial sensitivity test is a method to determine the susceptibility level of bacteria to antibacterial substances, to find out pure compounds that have antibacterial activity and to obtain potential antibacterial substances from natural products that have the ability to inhibit growth or kill bacteria at a low concentrations.[1]

Ethanol extract of mayana leaf (*Coleusatro purpureus L.*) had an antibacterial activity against *S. Aureus*.[3,4] The treatments of methanol extract of casturi stem with a concentration of 50% had an antibacterial effect on *S. aureus* of (13 mm) or greater compared to ampicillin (11 mm).

Another study,[5] stated that the antibacterial activity of the ethanol extract of Kopasanda leaf (*C. odorata L.*) had the highest activity in *Escherichia coli* bacteria which was 8.06 mm at 100% concentration, and n-hexane extract in *E. coli* bacteria was 8.45 mm at a concentration of 100%. Phytochemical screening results of the Kopasanda leaf extract (*C. odorata L.*) Ethanol solvents positively contain alkaloid, tanin and flavonoid compounds. In addition, studies on the leaf of the Tembelekan plant (*L. camara L.*) showed its has chemical contents including phenols, flavonoids and alkaloids.[6] The chemical content of Tembelekan (*L. camara L.*) has antibacterial activity against *Staphylococcus epidermidis*, and studies argued that the potency of Tembelekan leaf as wound medicine surpassed the potential of Bioplacenton drugs with faster healing indicators.[7] Therefore, researchers were interested in looking at the sensitivity of the Kopasanda and Tembelekan plants in inhibiting *S. aureus* bacteria.

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Materials and Methods

Type of Research

This is a laboratory experimental research with the design of The Static Group Comparison Group, which is by testing the inhibitory results of the extracts of Tembelekan leaf and Kopasanda leaf on the growth of *S. aureus* bacteria.

Plant materials

The sample used was obtained from the Kopasanda and Tembelekan wild plants,

which have often been used empirically as traditional medicine. The part taken was the leaf of the plant amounting to 500 gr.

Preparation of extracts

The extraction method that used in this study is the stratified extraction or maceration method (Quinn method, 1988) referred to in Darusman *et al*.[8] Tembelekan and Kopasanda leafs have been dried are stacked until they are smooth, then we soaked the macerated the leafs of the Tembelekan and Kopasanda into 1000 mL methanol for 24 hours. We separated the methanol extract from the leafs of Tembelekan and Kopasanda by filtering. Methanol extract was dried by evaporation of solvent.[5]

Antibacterial activity against *S. aureus*

S. aureus test bacteria were grown in liquid NB media and incubated at 37° C for 24 hours. Then the inhibitory power test was carried out using a dilution method to determine the minimum inhibitory content (MIC) of plant extracts against bacteria. The grown *S. aureus* was inoculated on Nutrient Agar media using the pouring method. The disc paper that contains extract of Kopasanda and Tembelekan leafs (at a concentration of 10%, 30%, 50% and 100%), positive (Ampicilin) and negative (Aquadest) controls were put on solidifies media. Afterwards it was incubated at 37° C for 24 hours.

The incubation period of the inhibition of Kopasanda and Tembelekan leafs can be seen by the formation of clear zones around the disc paper and measured by calipers. The time needed for incubation is 24 hours. The formula for inhibition as below.

$$\frac{(Dv - Dc) + (Dh - Dc)}{2}$$

Key: Dv = Vertical inhibition
Dc = Disk diameter
Dh = Horizontal Diameter (Torar *et al.*, 2015).

Results and discussion

The sensitivity of Tembelekan (*L. camara L.*) and Kopasanda (*C. odorata L*) leafs extract to *S. aureus* bacteria can be seen in Table 1.

From the results of statistical ANOVA test it emerged a significant difference between the activity of the extract of Tembelekan and Kopasanda leafs at a concentration of 100% with the positive control, this is evidenced by the value of sig. 0.000.

Tembelekan (*L. camara L*) is plant that grows wild in various places. The Tembelekan plant is used by the community traditionally to treat several types of diseases such as coughing, wounds, fever, swelling, gout and boils.[9] In this study, Tembelekan leafs extract was tested against *S. aureus* bacteria along with Kopasanda leafs extract. Kopasanda leafs are traditionally used as medicine in wound healing, as mouthwash for the treatment of sore throats, cough medicines, malaria medicine, antimicrobials, for headaches and as antidiarrheal.[10,11] Determination of antibacterial activity was carried out based on a comparison of the diameter of the inhibition zone that appeared around the disc paper which had been given an antibacterial substance in the form of test samples, namely Tembelekan leafs extract and Kopasanda leafs extract. The acquisition of methanol extract of Tembelekan leafs is by maceration. The liquid extract obtained is then continued with the evaporation process to assist the solvent evaporation process. The acquisition of thick extracts was carried out by evaporating the liquid extract resulting from evaporation with the help of the evaporator on the water bath. The thick extract obtained was gel-textured and chewy.

The inhibition test of methanol extract on Tembelekan leafs and Kopasanda leafs extract on *S. aureus* bacteria was carried out by growing test bacteria on agar media first. Then in the bacterial colony the paper discs are placed and incubated.

The results of the study summarized in Table 2 show that the Tembelekan leafs with a concentration of 100% were more sensitive than the Tembelekan leafs with concentrations of 10, 30% and 50% in inhibiting the growth of *S. aureus* bacteria. The inhibition zone formed by the concentration of 100% Kopasanda leafs extract is included in the medium category, while the inhibition zone formed by concentrations of 10%, 30% and 50% is in the weak category. Whereas, based on Table 2, it shows that the Kopasanda leafs extract with a concentration of 100% is more sensitive than the Kopasanda leafs extract with concentrations of 10% and 30%. The inhibition zone formed by a concentration of 50% and 100% of Kopasanda leafs extract is included in the medium category, while the inhibition zone formed by concentrations of 10% and 30% is in the weak category.[12] Based on the results of the data study, it is concluded that each concentration has different abilities to inhibit the growth of bacterial. According to previous studies[13] this occurs because the ability of an antimicrobial material to inhibit the living ability of microorganisms depends on the concentration of the anti-microbial material. The higher the concentration of the extract is, the higher the ability of the extract to inhibit microbial growth will be. In addition, according to, in addition to the concentration factor, the type of antimicrobial material also determines the ability to inhibit bacterial growth.

The relatively similar results of the study are shown in the research conducted[15] by which states that the extract of purslane herbs can strongly inhibit the *S. aureus* bacteria, and also showed the same results, that is, the ethanol extract of star fruit can inhibit Gram positive bacteria (*S. aureus*)

The test results of the research hypothesis based on Table 3 show the Sig 0.806>α (0.05) value, there is no significant difference in the sensitivity of Tembelekan leafs extract (*L. camara L.*) and Kopasanda leafs extract (*C. odorata L.*) to *S. aureus* bacteria. However, based on the average value of the

Table 1. The sensitivity of Tembelekan (*L. camara* L.) and Kopasanda (*C. odorata* L) leafs extract to *S. aureus* bacteria.

Leaf	Replication	Inhibitory zone (cm)					
		Extract				Control	
		10%	30%	50%	100%	(+)	(-)
Tembelekan	1	0	0	0.1	0.3	1	0
	2	0.1	0.3	0.5	1	1	0
	3	0.1	0.1	0.2	0.5	1	0
	Average	0.06	0.33	0.26	0.60	1	0
Kopasanda	1	0.5	0.2	0.5	0.9	1	0
	2	0.3	0.3	0.5	0.8	1	0
	3	0.2	0.5	0.5	0.7	1	0
	Average	0.3	0.3	0.5	0.8	1	0

Source: Primary Data 2018.

Table 2. Data normality test.

Leaf	n	Asymp. Sig. (2-tailed)
Tembelekan	4	0.991
Kopasanda	4	0.931

Table 3. ANOVA test.

Leaf	Std Deviation	Mean Square	F	Sig
Tembelekan	38.622	1491.667	0.000	0.000
Kopasanda	38.622	2137.500	10.688	0.211

inhibition zone, Kopasanda leaf extract was showed higher inhibition zone compared to Tembelekan leafs extract. This can be influenced by differences in components in the extract of Tembelekan and Kopasand leaf. Kopasanda leaf contains compounds eugenol, kavikol, allipyrokatekol and cavibetol which function as antiseptic substances that can inhibit bacterial growth. The researcher also tested the sensitivity of Tembelekan leafs and Kopasanda leafs extract to *S. aureus* bacteria using the well method. The test results of the well method showed that Kopasanda leafs extract was more sensitive to *S. aureus* bacteria compared to Tembelekan leafs extract where the inhibition zone formed by Kopasanda leafs was 0.85 cm while the inhibition zone formed by Tembelekan leafs extract was 0.70 cm.

The ability of Tembelekan and Kopasanda leafs extracts to inhibit the growth of *S. aureus* bacteria is caused by compounds or substances contained therein. According to previous studies [5] Tembelekan has many chemical constituents including essential oils, phenols, flavonoids, alkaloids, glycosides, iridoids glycosides, ethanoid phenyl, saponins, steroids, triterpins, sesquiterpenoids and tannins while the Kopasanda leafs contains several main compounds such as tannins, phenols, flavonoids, saponins and steroids. The results of the same study were carried out by stating that the giving of Kopasanda leaf extract had a major influence in inhibiting the growth of *Vibrio sp*. both on testing *in vitro* and *in vivo*. Whereas in another study[19] stated that the combination of the solution of Tembelekan leafs (*Lantana camara L.*) and papaya leafs (*Carrica papaya L.*) affected the death of aphids (*Aphis sp*.) on chili plants (*Capsicum annum L.*)

Conclusions

Based on the results of the study, it can be concluded that the extract of the Tembelekan and Kopasanda leafs can inhibit the growth of *Staphylococus aureus* bacteria, with results similar to the ampicillin antibiotic.

References

1. Soedarmo. Buku Ajar Infeksi dan pediatric tropis. Jakarta: Badan penerbit IDAI; 2008.
2. Jawetz E, Melnick JL, Adelberg EA, et al. Mikrobiologi Kedokteran. Edisi ke-20 (Alih bahasa: Nugroho & R.F.Maulany). Jakarta: Penerbit Buku Kedokteran EGC; 1995.
3. Mpila DA, Fatimawali, Wiyono WI. 2012. Uji Aktivitas Antibakteri Ekstrak Etanol Daun Mayana (Coleus atropurpureus L Benth) terhadap Staphylococcus aureus, Escherichia coli DAN Pseudomonas aeruginosa Secara In-Vitro. Jurnal Ilmiah Pharmacon. 2012;1:13-21.
4. Akbar MRV, Budiarti LY, Edyson. Perbandingan Efektivitas Antibakteri Antara Ekstrak Metanol Kulit Batang Kasturi Dengan Ampisilin Terhadap Staphylococcus Aureus in Vitro. Berkala Kedokteran. 2016;12:1-9.
5. Sukarno. Uji Aktivitas Antibakteri Ekstrak Etanol, Etil Asetat dan n-Heksana Daun Laruna (Chromolaena Odorata L) Terhadap Bakteri Staphylococcus Aureus dan Escherichia Coli. Degree Diss., Universitas Islam Negeri Alauddin Makassar, Indonesia; 2017.
6. Parwanto MLE, Senjaya H, Edy HJ. Formulasi Salep Antibakteri Ekstrak Etanol Daun Tembelekan (Lantana camara L). Pharmacon Jurnal Ilmiah Farmasi. 2013;2:104-108.
7. Rijai L. Potensi Tumbuhan Tembelekan (Lantana camara Linn) Sebagai Sumber Bahan Farmasi Potensial. J Trop Pharm Chem 2014;2:192-247.
8. Darusman LK, Sajuti D, Komar, Pamungkas. Ekstraksi komponen bioaktif sebagai obat dari kerang-kerangan, bunga karang dan ganggang laut di perairan pulau pari kepulauan seribu. Buletin kimia. 1995;2:41-60.
9. Dalimartha S. Atlas Tumbuhan Obat Indonesia. Jilid I. Trubus Agriwidya. Anggota IKAPI. Jakarta: PT. Pustaka Pembangunan Swadaya Nusantara; 1999.
10. Yenti SR. Kinetika Proses Pembuatan Asam Oksalat dari Ampas Tebu. Pekanbaru: Jurnal SNTK Topi. 2011;29-32.
11. Hadiroseyani Y, Hafifuddin, Alifuddin M, Supriyadi H. Potential of Chromolaena odorata Leaf as A Cure of Aeromonas hydrophila on Giant Gouramy. Jurnal Akuakultur Indonesia. 2005;4:139–144.
12. Schlegel HG. Mikrobiologi Umum. Penterjemah: Tedjo Baskoro. 6th ed. Yogyakarta: Gajah Mada University Press; 1994.
13. Davis WW, Stout TR. Disc plate methods of microbiological antibiotic assay. Appl Microbiol. 1971; 659-665.
14. Ajizah A. Sensitivitas Salmonella typhimurium terhadap ekstrak daun Psidium guajava L. Bioscientiae. 2004; 1:31-38.
15. Hermawan A. Pengaruh Ekstrak Daun Sirih (Piper betle L.) Terhadap Pertumbuhan Staphylococcus aureus Dan Escherichia coli Dengan Metode Difusi Disk. Degree Diss., Universitas Airlangga, Indonesia; 2007.
16. Lathifah QA. Uji Efektifitas Ekstrak Kasar Senyawa Antibakteri Pada Buah Belimbing Wuluh (Everrhoa bilimbi L.) Dengan Variasi Pelarut. Degree Diss., Universitas Islam Negeri Maulana Malik Ibrahim, Indonesia; 2008.
17. Jabarsyah A, Rugian D, Arniati. Pengaruh Ekstrak Daun Sirih Terhadap Pertumbuhan (Vibrio sp). Jurnal Harpodon. 2009; 2:24-30.
18. Manguntungi B, Kusuma AB, Yulianti, et al. Pengaruh Kombinasi Ekstrak Kirinyuh (Chromolaena odorata) dan Sirih (Piper betle L) dalam Pengendalian Penyakit Vibriosis pada Udang. Biota. 2016;1:138-144.
19. Erlangga JWW, Danuji S, Anitasari SD. Pengaruh Kombinasi Daun Tembelekan (Lantana camara L.) Dan Daun Pepaya (Carica papaya L.) Sebagai Biopestisida Terhadap Hama Kutu Daun (Aphis sp.) Pada Tanaman Cabai (Capsicum annum L.). National Seminar Proceedings on SIMBIOSIS II, 2017 September 30, Madiun, Indonesia.