

Dental caries bacterial isolates sensitivity toward water-, ethanolic-extract and essential oils of several shallot cultivars (*Allium cepa*)

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Abstract. *Indrawati I, Anggraini AP, Irawan B. 2017. Dental caries bacterial isolates sensitivity toward water-, ethanolic- extract and essential oils of several shallot cultivars (Allium cepa). Nusantara Bioscience 9: 229-235.* Sensitivity test of dental caries bacterial isolates has been carried out toward water-, ethanolic- extract and essential oil of Batu, Maja, Sumenep shallot cultivars. The experimental method was conducted using sensitivity test through Kirby-Bauer method with randomized complete design factorial 5 x 16 for each shallot cultivars with two replications. Factor one (i) was dental caries bacteria isolates (*Lactobacillus* sp., *Staphylococcus* sp.1, *Staphylococcus* sp.2, *Staphylococcus* sp.3 and *Streptococcus* sp.). The other factor (ii) was an antibacterial agent (water-extract, ethanolic- extract and essential oil of Batu, Maja, Sumenep shallot cultivars and antibiotic Vancomycine 30 µg). Concentration for each shallot cultivars extract that used was 80%, 40%, 20%, 10% and 0%. The result of sensitivity test to those antibacterial agents showed that 80% water extract of Maja cultivar had the best inhibition zone of *Staphylococcus* sp.1 growth with the average of inhibition zone 37 mm. The interaction factor between essential oil from Batu and Maja as well as Sumenep shallot cultivar revealed a better inhibition zone than Vancomycine 30 µg with all bacterial isolates tested.

Keywords: Dental caries, *Lactobacillus*, shallot, *Staphylococcus*, *Streptococcus*

INTRODUCTION

Dental caries is a disease caused by the interaction between bacterial plaque, diet, and teeth. Plaque dominated by *Streptococcus mutans* and *Lactobacillus* sp. is the cariogenic bacteria due to their ability in forming acid from fermented carbohydrate. These bacterial well grew in an acidic atmosphere and attached to the dental surface due to their ability in producing extracellular polysaccharides. These extracellular polysaccharides consist of glucose polymer that causing plaque matrix with gelatinous consistency, thus facilitate bacteria to stick to the teeth and attach to each other, thus inhibiting saliva to carry its antibacterial activity (Pratiwi 2005).

Bacterial that has a significant role in plaque formation is the bacteria that able to ferment polysaccharides (carbohydrate) extracellular, for instance, the bacteria from the genus of *Streptococcus*, *Staphylococcus* and *Lactobacillus* (Kleinberg 2002). These bacteria are able to ferment carbohydrate and produce organic acid thus change the pH of the buccal cavity into acidic. Indeed, the structure of dental email will be dissolved in pH < 5.41 and provoke dental caries (Hutagalung 2004). Plaque accumulation prevention can be performed by diet selection and clean the tooth regularly either with toothpaste or mouth washer. Toothpaste that is widely distributed in the market contains fluor that has an antibacterial effect. However, toothpaste with a high fluor concentration may give a secondary effect such as email fluorosis and relatively ineffective due to its ability as inhibitor bacterial growth instead of exterminating them (Dea 2006). Antibiotics treatment to

overcome plaque such as Peniclyline, Vancomycine , and Chlorohexydine can provide the resistance and secondary effect, for instance, dental discolorization (van Winkelhoff et al. 2000). To date, an increased attention has been paid to the use of antibacterial agent from a natural product. The advantage of using this natural product is suppressing the secondary effect produced compared to synthetic antibacterial. One of the potential natural antibacterial agents is the shallot (*Allium cepa* L.). In West Java, there are eight shallot cultivars of which, Batu, Maja and Sumenep cultivar that are considered as the most produced cultivars in this region (Irawan et al. 2004). These cultivars have an essential oil that composed by several antibacterial sulfide compounds (Arnelia 2004; Wahyu 2005). Sulfide compounds in the shallot can destroy oral bacteria including *Streptococcus mutans*, the cause of dental caries, organosulfur compounds were cited as protective agents by researchers finding antibacterial effects of onion extract against oral pathogenic bacteria (Kim 1997). In this study, essential oil from the three different shallot cultivars (Batu, Maja and Sumenep) was investigated. These three cultivars have essential oil with high content of sulfide compounds compared to other cultivars (Wahyu 2005). Indeed, the three shallot cultivars have different traits regarding morphology and also chemical properties. Bulbs layer of Batu shallot cultivars, for instance, have a single and clustered red bulb layer. This Batu cultivar is containing disulfide-type of essential oil compounds, trisulfide, thiophene, thiosulfinate, furan and hydrocarbon. As for Maja cultivar, it is characterized by its dark red and clustered bulbs layer. This cultivar has almost similar

chemical properties except for the absence of thiosulfinate that is replaced by mercaptan. In contrast to other cultivars, bulbs of Sumenep shallot cultivar have a clustered elongated oval-shaped with pale red color. This cultivar also containing disulfide-type of essential oil compounds, trisulfide, thiophene, sulphone, mercaptan, furan, sulfuric acid and hydrocarbon (Irawan et al. 2004; Putrasamedja 1996; Wahyu 2005).

Sensitivity test of dental caries bacterial isolates was performed using water- and ethanolic- extract from these three cultivars. Shallot extraction with different solvent was used to bind the secondary metabolite in shallot itself. Water and ethanol solvent were used due to their difference in polarity and contain secondary metabolite extracted material (Harborne 1998). Moreover, the phytochemical test was carried out to identify the difference in antibacterial activity on each shallot extracts. Apart from water-, ethanolic- extract, and essential oil, a synthetic antibiotic Vancomycine 30 µg was also used as a comparator. Use of Vancomycine and shallot in dental caries has a similarity on their usage, which is they have a direct contact with dental caries. However, a clear distinction should be made between the Vancomycine and shallot in terms of the usage. Vancomycine is the antibiotic that is generally available in mouth washer or toothpaste, whereas shallot is normally chewed directly by a tooth with dental caries in traditional medicine (Caufield et al. 2001).

MATERIALS AND METHODS

This study using a descriptive and experimental method in the laboratory. Descriptive method was used in isolating, identifying dental caries bacterial isolates, shallot extraction, phytochemical test and minimum inhibition concentration test. Whereas, experimental method was used in bacterial sensitivity test in Kirby-Bauer paper disc (Hudzicki 2009). The observed parameters were the transparent zone formation in the paper disc, which means the bacterial growth inhibition or the lethal zone.

Experimental research in this study was randomized complete design factorial 5 x 16, with two replications were provided for each shallot cultivars. The first factor (i) was dental caries bacteria isolates (*Lactobacillus* sp., *Staphylococcus* sp.1, *Staphylococcus* sp.2, *Staphylococcus* sp.3 and *Streptococcus* sp.). While the other factor (ii) was the antibacterial agent (water-, ethanolic- extract and essential oil of Batu, Maja, Sumenep shallot cultivars and antibiotic Vancomycine 30 µg). Concentration for each shallot cultivar extract that used is 80, 40, 20, 10 and 0%. Data obtained was statistically analyzed by using Analysis of Variance (ANOVA test). When the difference between treatment was observed, Duncan test was performed to identify the difference in means from each treatment with 95% of confidence ($\alpha = 0.05$).

Dental caries bacterial isolation and identification

Bacterial isolates were taken from dental caries patients which consist of three males and three females due to the technical limitations. Furthermore, samples were inserted

into the vial bottle containing liquid broth, then were incubated at 37°C for 24 h. Series of dilution range from 10^{-1} - 10^{-6} were made from the turbid liquid medium. From the last three dilutions, 1 mL of each was taken and then were inserted into a sterile petri dish. Subsequently, 20 mL of nutrient agarose were added, and the mixture was incubated at the similar condition as mentioned above. The macroscopic observation was performed when the bacteria grow on the agarose plates. In addition, each bacterial colony that represents the difference was then isolated in the slope agarose. Pure isolates were identified by using the Bergey's Manual of Determinative Bacteriology (Whitman et al. 2012).

Shallot cultivar extraction

Shallot cultivar of Batu, Maja and Sumenep were extracted using maceration method with ethanol, then mixed for 24 h. Afterward, extracts were separated and were then submerged with ethanol for 24 h. Extracts were then re-separated, and shallot submersion was performed three times in 24 h. Moreover, extracts from submersion were evaporated. A similar process was conducted using sterilized deionized water to obtain extract water. The essential oil was obtained by Stahl distillation (Harborne 1998). The distillate was then mixed with hexane solvent and filtered. The mixture between distillate and solvent was allowed to stand until two different layers were formed. Extracts were then added with Mg_2SO_4 in order to bind water and then filtered by using filter paper. Final essential oil was then evaporated at 37-40°C.

Phytochemical test

The phytochemical test was performed by a precipitation reaction and color enhancement (van Winkelhoff et al. 2000) or instance alkaloid, flavonoid, saponins, tannin, steroid, terpenoid, monoterpene, sesquiterpenoid, quinone, and phenol.

Minimum inhibition concentration (MIC) test

MIC was performed using several shallot extract concentration by tube dilution method with two replicates. Extract was made in several concentrations: 80, 40, 20, 10, 5, 2.5, 1.25, 0.625% and control (without shallot extract addition). Each shallot extract was diluted using a solvent that corresponds to the type of extract (Prabuseenivasan et al. 2006; Setzer et al. 2004; Bruni et al. 2004). One ose of obtained bacterial pure culture was then suspended into sterile NaCl physiologic with 0.5 Mac Farland turbidity. This bacterial suspension was then taken (1 ose) and was inserted into a reaction tube containing 1 mL shallot extract with different concentration and 1 mL of liquid broth as a bacterial growth medium. This mixture was then suspended and incubated in 37°C for 24 h.

Sensitivity test of dental caries bacterial isolates on shallot extract

Sensitivity test of dental caries bacterial isolates on shallot extract using water-, ethanolic- extract and antibiotic Vancomycine 30µg was conducted using paper disc method with two replicates. Shallot extract

concentration used was the concentration above and below the MIC. Paper discs were submerged in varying shallot extracts with different concentrations for 24 h, and were then dried. Bacterial isolates were suspended using sterilized physiologic NaCl with 0.5 Mac Farland turbidity. Then 1 mL of bacterial suspension was taken and inserted into sterilized petridish and 20 mL of Mueller Hinton agarose was added, let stand till frozen. A paper disc containing the extract was the placed on the surface of the medium. Sensitivity test results were then analyzed after 24 h of incubation in 37°C.

RESULTS AND DISCUSSION

Dental carries bacterial isolates

Three bacterial genera were identified from bacterial isolates obtained from 6 samples of dental caries patients (3 males and 3 females). These three bacterial genera are *Lactobacillus*, *Staphylococcus*, and *Streptococcus*. They are commonly found in dental caries (Table 1).

The human mouth can contain a large variety of microbes—as many as 31 distinct microbes have been reported (Ajizah 2004). More typically, 14 varieties make up the normal microbial flora of the mouth. These microbes were *Streptococcus* spp., *Staphylococcus aureus*, *Candida albicans*, and a yeast-like fungal species. The number of plates on which they grew was relatively few, generally, and specifically minimal with respect to each species (Okeke 2003).

Extraction of different shallot cultivars

From extraction of 1 kg wet weight with water solvent, Batu cultivar produced 97 g of extract, while Maja and Sumenep cultivars were 88.64 and 70 g, respectively. Essential oil obtained from each shallot cultivar through Stahl distillation was only 2 mL. The result yielded from the extraction of different shallot cultivar was only a few, due to high water content (88% from the initial wet weight).

Phytochemistry of shallot extracts

Phytochemical test by the color reaction and precipitation methods showed that different secondary metabolites were observed on each shallot extract (Table 2).

The antimicrobial and antifungal activities were attributed to the presence of alkaloids, tannins, saponins, flavonoids, cardiac glycosides, and anthraquinones (Dabur et al. 2007). Onions have a unique combination of three families of compounds that are believed to have salutary effects on human health, i.e., fructans, flavonoids and organosulfur compounds. Fructans are small carbohydrate molecules that help to maintain gastrointestinal health by sustaining beneficial bacteria. A great deal of research has focused on one flavonoid, quercetin, which is found at particularly high levels in onions. It functions as an antioxidant, deactivating molecules that are injurious to cells in the body. Onion oil has been shown to be highly effective against the growth of gram-positive bacteria, dermatophytic fungi and aflatoxin production of *Aspergillus* fungi genera (Carroll et al. 2013).

Table 2. Phytochemical test of water and ethanol extract several shallot cultivars

Test type	Shallot extract					
	Water extract			Ethanol extract		
	Batu	Maja	Sumenep	Batu	Maja	Sumenep
Alkaloid						
Mayer	-	+	-	-	+	-
Hager	+	+	+	+	-	+
Dragendroff	-	-	-	-	-	-
Flavonoid	-	+	+	-	+	+
Saponin	-	+	-	-	+	+
Tannin	-	-	-	-	-	-
Steroid	+	+	-	-	-	-
Quinone	+	+	+	+	+	+
Phenol	+	+	+	+	+	+
Terpenoid	+	+	+	+	+	+
Monoterpenoid	+	+	+	+	+	+
Sesquiterpenoid	+	+	+	+	+	+

Note: + = present, - = absent

Table 1. Dental caries bacterial isolates species from dental caries patient

Identification test	Bacterial species				
	<i>Lactobacillus</i> sp.	<i>Staphylococcus</i> sp. 1	<i>Staphylococcus</i> sp. 2	<i>Staphylococcus</i> sp. 3	<i>Streptococcus</i> sp.
Gram coloration	Gram positive (+)	Gram positive (+)	Gram positive (+)	Gram positive (+)	Gram positive (+)
Cell form	Long bacillus	Spherical	Spherical	Spherical	Spherical
Colony form	Spherical, flat edges	Spherical, uneven edges	Spherical, uneven edges	Spherical, uneven edges	Spherical, uneven edges
Colony surface	Flat	Convex	Convex	Convex	Convex
Colony color	White yellowish	White turbid	White	White yellowish	White
Spores coloration	No spores	No spores	No spores	No spores	No spores
O ₂ requirement	Facultative anaerobic	Facultative anaerobic	Facultative anaerobic	Facultative anaerobic	Facultative anaerobic
Motility	Nonmotile	Nonmotile	Nonmotile	Nonmotile	Nonmotile
Catalase test	Negative (-)	Positive (+)	Positive (+)	Positive (+)	Negative (-)
Glucose test	Positive (+)	Positive (+)	Positive (+)	Positive (+)	Positive (+)

Note: + = Positive reaction, - = Negative reaction

MIC test of several shallot cultivars on dental caries bacterial isolates

Extract of several shallot cultivars with water solvent produced the best MIC. This perhaps due to several dental caries bacterial isolates with low MIC (1.25%) in water-extract Sumenep shallot cultivar. In contrast, the ethanolic-extract and essential oil of shallot produced 5% of MIC in average. MIC test showed that five bacteria tested were unable to grow at 10% of concentration, thus sensitivity test was performed from concentration 10%.

Sensitivity of dental caries bacterial isolates on antibiotic Vancomycine

In this study, 30 µg of Vancomycine was used to compare the sensitivity of tested bacteria on different shallot extracts (Table 3). Carroll et al. (2013) observed that Vancomycine has bactericidal effect by inhibiting biosynthesis of bacterial cell wall by disrupting cell wall permeability. Additionally, Vancomycine can also trigger RNA synthesis in bacteria.

Sensitivity dental caries bacterial isolates on water- and ethanolic- extract as well as essential oil shallot Batu cultivar

ANOVA showed that there was an effect from bacterial species, antibacterial agents (water- and ethanolic- extract, essential oil of Batu shallot cultivar and Vancomycine 30 µg) and interaction between the two factors on the diameter of inhibition zone at 1% of confidence. Therefore, subsequence test was conducted with Duncan multiple tests on the bacterial interaction and antibacterial agents.

Duncan test showed that interaction between essential oil of Batu shallot cultivar with concentration 80% and *Lactobacillus* sp. has the highest inhibition (26 mm). The interaction between the essential oil of Batu's cultivar with concentration 80% on *Lactobacillus* sp., *Staphylococcus* sp.1, *Staphylococcus* sp.2 and *Streptococcus* sp., and essential oil with concentration 40% on *Streptococcus* sp. produced a better diameter of inhibition zone than Vancomycine 30 µg with all bacterial tested.

Duncan test displayed that there were strongly very sensitive, strongly sensitive and semi-sensitive tested bacteria. This perhaps due to the difference in morphological characteristics of bacteria tested (Ajizah 2004). Hartini et al. (2002) demonstrated that the difference in inhibition zone on each bacterial isolates could be due to the bacterial cell wall structure that has particular selectivity in bioactive absorption.

Inhibition zone produced by the essential oil of shallot Batu cultivar was higher than the water- and ethanolic-extracts. This could be due to the essential oil content in Batu cultivar has better ability in inhibiting the growth of dental caries bacterial isolates compared to other secondary metabolites, which contained in water- and ethanolic-extracts. Shallot of Batu cultivar contained essential oil such as disulfide, trisulphide, thiophene, thiosulfinate, phurane and hydrocarbon. This result was affirmed by

(Stout 2001) and Ajizah (2004) who revealed that essential oil can inhibit the growth of bacteria or acts as a bacterial disinfectant by disrupting membrane formation or cell wall thus these two components are undeveloped. Additionally, the types of secondary metabolite in water- and ethanolic-extracts of Batu cultivar are less than other shallot's cultivar.

Sensitivity of dental caries bacterial isolates on water and ethanol extracts and essential oil of shallot Maja cultivar

ANOVA showed that there was an effect from bacterial species, antibacterial agents and the interaction between those two, on the diameter of inhibition zone with 1% of confidence. Therefore, supplementary test with Duncan test was only performed on the bacterial interaction and antibacterial agents.

Duncan test revealed that interaction between water extract of shallot Maja cultivar concentration 80% on *Staphylococcus* sp.1 had the highest the average of inhibition zone (37 mm). In contrast, the interaction of water-extract Maja shallot cultivar 80% and 40% of tested bacteria demonstrated a better diameter of inhibition zone than Vancomycine 30 µg. Similar to previous results, categorization on tested bacterial characteristics was made according to (Schlegel 1993). This is due to the different selectivity of the bacterial cell wall to bioactive transport (Robinson 1983).

The phytochemical test revealed that water-extract of Maja shallot cultivar containing more alkaloids than its ethanolic- extract, based on the positive reaction occurred on alkaloid reactor. Alkaloid has antibacterial effect by inhibiting bacterial cell wall formation. Deceleration on bacterial cell wall synthesis will affect the strength of cell wall and provokes lysis in bacterial cell. Additionally, essential oil contains volatile component thus the ability of essential oil in inhibiting bacterial growth is not maximal.

The sensitivity of dental caries bacterial isolates on water- and ethanolic- extracts as well as essential oil of Sumenep shallot cultivar ANOVA showed that significant effect was observed in antibacterial agents used on the diameter of inhibition zone, but no interaction was found.

Table 3. Sensitivity test of dental caries bacterial isolates on antibiotic Vancomycine 30 µg

Bacteria	Category ^{*)}			
	Average	R	I	S
<i>Lactobacillus</i> sp.	21.00			√
<i>Staphylococcus</i> sp. 1	19.50			√
<i>Staphylococcus</i> sp. 2	20.50			√
<i>Staphylococcus</i> sp. 3	19.50			√
<i>Streptococcus</i> sp.	21.00			√

Note: *) According to the standard interpretation diameter of inhibition zone based on the NCLLS (2003) and Koneman et al. (2001). R = Resistant; I = Intermediate/ semi sensitive; S = Sensitive

Table 4. Duncan test on interaction between bacterial species and antibacterial agents (water- and ethanolic- extracts, essential oil and Vancomycine 30 µg) on the diameter of inhibition zone on Batu shallot cultivar

Antibacterial agents (b)	Bacterial species (a)				
	a1	a2	a3	a4	a5
	<i>Lactobacillus</i> sp.	<i>Staphylococcus</i> sp. 1	<i>Staphylococcus</i> sp. 1	<i>Staphylococcus</i> sp. 3	<i>Streptococcus</i> sp.
	Average of Inhibition zone diameter (mm)				
Ethanol extract shallot Batu cultivar	13.5 bc	13 bc	7.5 a	15.5 c	9 a
concentration b1 (80%)	BC	BCD	A	C	A
b2 (40%)	11 b	9.5 ab	7 a	9.5 ab	7.5 ab
	BC	A	A	AB	A
b3 (20%)	10 a	7 a	7 a	8.5 a	7 a
	ABC	A	A	AB	A
b4 (10%)	9 a	7 a	7 a	8.5 a	7 a
	ABC	A	A	AB	A
b5 (0%)	6 a	6 a	6.5 a	6 a	6 a
	A	A	A	A	A
Water extract shallot Batu cultivar	12 b	11 ab	8.5 ab	7.5 a	7 a
concentration b6 (80%)	G	F	F	A	A
b7 (40%)	11 a	11 a	8.5 a	7.5 a	7 a
	F	E	E	A	A
b8 (20%)	10 a	10 a	7 a	7 a	7 a
	F	E	CD	A	A
b9 (10%)	9.5 a	10 a	7 a	7 a	7 a
	DE	D	BC	A	A
b10 (0%)	6 a	6 a	6.5 a	6 a	6 a
	A	A	A	A	A
Essential oil shallot Batu cultivar	26 d	21.5 bc	22.5 bcd	12.5 a	23.5 cd
Concentration b11 (80%)	E	CD	E	BC	B
b12 (40%)	20.5 b	9.5 a	21 b	11.5 a	21.5 b
	DE	B	DE	AB	B
b13 (20%)	16.5 b	7 a	20 b	9.5 a	9.5 a
	CD	A	BC	AB	A
b14 (10%)	13.5 b	7 a	12 b	7 a	7.5 a
	ABC	A	B	A	A
b15 (0%)	7 a	7 a	7 a	7 a	7 a
	AB	A	A	A	A
Vancomycine 30 µg b16	21 a	19.5 a	20.5 a	19.5 a	21 a
	F	BC	DE	D	B

Note: Comparison of the average value that is followed by similar uppercase letter towards column direction and lowercase letter towards row direction show no significant difference observed on Duncan test with 1% of confidence

Duncan test revealed that water extract with concentration 80% had the highest inhibition zone (27.200 mm). In contrast, water-extract with concentration 0% exhibited the lowest inhibition zone (6 mm). Whereas water-extract with concentration 80% produced a better inhibition zone than antibiotic Vancomycine 30 µg.

Water- and ethanolic- extracts of Sumenep shallot cultivar contain alkaloid. Alkaloid has antibacterial effect by disrupting bacterial cell wall formation. Additionally, ethanolic- extract contains saponins that unavailable in water-extract of Sumenep shallot cultivar. Saponin has antibacterial activity by deteriorating bacterial cell wall permeability (Robinson 1983). Moreover, water- and ethanolic- extracts contain flavonoid which is also able to hamper cell wall permeability. Ethanolic- extract contains more types of secondary metabolite but unable to provide a better antibacterial activity compared to water-extract. This probably due to secondary metabolites that are available in water-extract is more abundant quantitatively compared to

the ethanolic- extract of Sumenep shallot cultivar.

In summary, dental caries bacterial isolates are sensitive to water- and ethanolic- extracts, as well as essential oil of Batu, Maja and Sumenep shallot cultivars. Water- and ethanolic- extracts, essential oil of Batu, Maja and Sumenep shallot cultivar as well as antibiotic as a comparison, have different antibacterial activity. Interaction of essential oil of Batu shallot cultivar concentration 80% with *Lactobacillus* sp., *Staphylococcus* sp.1, *Staphylococcus* sp.2 and *Streptococcus* sp., essential oil of Batu cultivar 40% with *Streptococcus* sp., water-extract of shallot Maja cultivar 80% and 40% with tested bacterial and water-extract of Sumenep shallot cultivar 80% had better inhibition zone compared to Vancomycine 30 µg on all tested bacterial. In general, water- and ethanolic- extract of Batu, Maja and Sumenep shallot cultivar have secondary metabolites such as alkaloid, flavonoid, saponins, steroid, quinone, phenol, terpenoid, monoterpenoid dan sesquiterpenoid.

Table 5. Duncan test on the effect of interaction between bacterial species and antibacterial agents (water and ethanol extract, essential oil and Vancomycine on diameters of inhibition zone in shallot Maja cultivar

Antibacterial agents (b)	Bacterial species (a)				
	a1 <i>Lactobacillus</i> sp.	a2 <i>Staphylococcus</i> sp. 1	a3 <i>Staphylococcus</i> sp. 2	a4 <i>Staphylococcus</i> sp. 3	a5 <i>Streptococcus</i> sp.
	Average of Inhibition zone diameter (mm)				
Ethanol extract shallot Maja cultivar concentration b1 (80%)	11 a	20 c	9 a	8 a	16.5 b
b2 (40%)	BC	BC	A	A	CD
	10.5 a	8.5 a	8 a	7.5 a	11 a
	BC	A	A	A	B
b3 (20%)	9 a	7 a	8 a	7 a	7 a
	ABC	A	A	A	A
b4 (10%)	8.5 a	7 a	7 a	7 a	7 a
	ABC	A	A	A	A
b5 (0%)	6 a	6 a	6,5 a	6 a	7 a
	A	A	A	A	A
Water extract shallot Maja cultivar concentration b6 80%	26.5 a	37 b	28.5 a	26 a	27.5 a
	H	E	G	E	F
b7 (40%)	23 a	32 b	23 a	22 a	21 a
	GH	D	F	D	E
b8 (20%)	21.5 b	29 c	17.5 a	17.5 a	20 ab
	G	D	DE	C	DE
b9 (10%)	15.5 a	23.5 b	16.5 a	13.5 a	14 a
	DE	C	CD	B	BC
b10 (0%)	6 a	6 a	6 a	6 a	6.5 a
	A	A	A	A	A
Essential oil shallot Maja cultivar concentration b11 80%	17.5 ab	21 bc	23.5 c	14 a	29 d
	EF	BC	F	BC	F
b12 (40%)	15 b	17.5 bc	20.5 c	9 a	27 d
	DE	B	E	A	F
b13 (20%)	12.5 bc	10 ab	15.5 c	7 a	26.5 d
	CD	A	BC	A	F
b14 (10%)	10 ab	9 ab	12.5 b	7 a	17 c
	ABC	A	B	A	DE
b15 (0%)	7 a	6.5 a	6.5 a	7 a	6.5 a
	AB	A	A	A	A
Vancomycine 30 µg b16	21 a	19.5 a	20.5 a	19.5 a	21 a
	FG	B	EF	CD	E

Note: Comparison of the average value that is followed by similar uppercase letter towards column direction and lowercase letter towards row direction show no significant difference observed on Duncan test with 1% of confidence

Table 6. Duncan test on the effect of antibacterial agents on diameters of inhibition zone of shallot Sumenep cultivar ($\alpha = 0,01$)

Antibacterial agents	Average of inhibition zone diameter (mm)
Water extract 0%	6.00 a
Ethanol extract 0%	6.70 ab
Essential oil 0%	6.90 ab
Ethanol extract 10%	7.40 ab
Ethanol extract 20%	7.90 abc
Essential oil 10%	8.20 abc
Essential oil 20%	9.30 bcd
Ethanol extract 40%	9.60 bcd
Essential oil 40%	10.80 cd
Ethanol extract 80%	11.70 de
Essential oil 80%	13.80 ef
Water extract 10%	14.50 ef
Water extract 20%	16.40 f
Vancomycine 30 µg	20.30 g
Water extract 40%	23.00 g
Water extract 80%	27.20 h

Note: Similar letter towards column direction showed no significant difference

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