https://dx.doi.org/10.22161/ijaers.4.7.14 *ISSN: 2349-6495(P) | 2456-1908(O)*

The Effect of various Sterilization Procedure on Number of Colony-Forming units of isolated endophytic bacteria from *Cosmos caudatus* Kunth. leaf

Fiqih Ramadhan, Fikri Ainur Risma Hardianti Oktavia, Luluk Mukarramah, Ria Yulian, Nurul Hilyatun, Iis Nur Asyiah

Biology Education, Faculty of Teacher Training and Education, University of Jember, Indonesia

Abstract— *The purpose of this study was to determine the effect of different concentration and time sterilization on the number of colony-forming units of isolated endophytic bacteria from Cosmos caudatus kunth. leaf. The sterilizing agents were used is sodium hypochlorite (NaOCl). The research consisted of four treatments P1 (NaOCl 5.35%; 3 minutes), P2 (NaOCl 5.35%; 1,5 minutes), P3 (NaOCl 3%; 3 minutes), and P4 (NaOCl 3%; 1,5 minutes). Every treatments have one control medium and three repetition. The control medium treated by make streak using C. caudatus leaf that had sterilized and the three repetition treated by inoculating 100 µL suspension of C. caudatus leaf with 10^{-1} serial dilution. Result on the control medium is sterile and indicated that the bacteria in treatments medium is endophytic bacteria. The CFU in treatments P1 ($0x10^2$ CFU/ml), P2 ($2,9 X 10^3$ CFU/ml), P3 ($2,9 X 10^3$ CFU/ml), and P4 ($2,8 X 10^3$ CFU/ml). The conclusion from this study is there was no significantly different of different concentration and time sterilization on the number of colony-forming units of isolated endophytic bacteria from Cosmos caudatus kunth. Leaf.*

Keywords— Sodium hydroxide, endophytic bacteria, leaf Cosmos caudatus.

I. INTRODUCTION

Cancer or carcinoma is a group of diseases characterized by the growth and development of uncontrolled and abnormal cells. Nowadays, cancer is one of the main threats and causes of death for human. According to data from the IARC in 2012, cancer has been the cause of death of 8,201,575 people worldwide. While in Indonesia cancer rates by 1.4% (Ministry of Health Republic of Indonesia. 2015). In cancer, the cancer cells can multiply and spread to the far location from the origin place This is what causes the cancer becomes deadly disease. Current cancer treatment was assessed to be less secure. Surgery, chemotherapy, radiation therapy and others still have severe side effects. Therefore one of alternative cancer treatment is to use natural ingredients that can reduce the side effects of cancer treatment today.

Cosmos caudatus Kunth. is one of the plants that are considered to have anticancer agents and has been widely used in traditional medicine. *C. caudatus* Kunth produce one form of secondary metabolites flavonoids thatassessedcan be used as an anticancer agent., Existing research results showed *C. caudatus* Kunth leaves contain antioxidant compounds are quite high at 70 mg/L (Lotulung et al., 2001). In another study, methanolic extract of *C. caudatus* Kunth leaves contain flavonoids and quercetin glycosides (Abas et al., 2003). This makes the plant *C. caudatus* Kunth can be used as drugs in the treatment of cancer. Research on the use of *C. caudatus* Kunth as a cancer drug has been widely reported among others as triggers apoptosis (programmed cell death) (Ren et al., 2003); can induce apoptosis of colon cancer cells, and leukemia (Taraphdar, 2001); and also induces apotosis in breast cancer cells (Pebriana, etal.,2008).

Utilization *C. caudatus* Kunth as an anticancer drug is still in the laboratory scale. For large-scale use (industry), the use of *C. caudatus* Kunth or other plants as anticancer plant drugs still have limitations. This is because if it is produced as an herbal medicine on an industrial scale will cause the limitations of raw materials because most of the raw materials of herbal medicine taken from the mother plant. So it is feared that these biological resources will be destroyed due to the constraint in cultivation. In *C. caudatus* Kunth plant itself has a life cycle that is greatly influenced by the environment, it will affect the growth of plants *C. caudatus* Kunth including the leaves. Besides, *C. caudatus* Kunth are not able to produce a compound which is pretty much in a relatively short time.

Sterilization of plant organs that will be used as materials for endophytic bacteria isolation becomes very important. This is because the sterilization stage determines whether the bacteria isolated are plant endophytic bacteria or are

https://dx.doi.org/10.22161/ijaers.4.7.14 *ISSN: 2349-6495(P) | 2456-1908(O)*

contaminant bacteria attached to the surface of the plant organ. Sodium hypochlorite (NaOCl) is one of the most commonly used materials in surface sterilization. This material is widely contained in commercial whitening products such as *Fast* and *Clorox* and can be diluted according to the desired concentration (Mahmoud and Al-Ani, 2016). The concentration of NaOCl used for surface sterilization is different for each plant organ and also different for each plant species. In addition to the concentration factor, the duration of sterilization also determines the successful isolation of endophytic bacteria. Sterilization time difference is determined by the morphology of each organ and plant species. For hard plant organs such as a rod needs a longer time than leaves. The improper concentration and duration of sterilization will cause tissue damage to plants that can cause endophytic bacteria that are located on the tissue become die.

The present study aimed to present the effective sterilization procedure in both the NaOCl concentration and the duration of sterilization on the number of colonies resulting from endophytic bacterial isolation from the leaves of the *C. caudatus* Kunth

II. METHODOLOGY

Cosmos caudatus Kunth. plants obtained from Tidar Hill, Jember, East java, Indonesia. The leaf excised from health and maximally growth of 3rd-5th leaf, that growth from the top of shoot.

In this study variation of sterilization methode that used are different concentrations and time exposure. The sterilizing agents was use is, sodium hypochloride (NaOCl) which contain (5,25%) NaOCl as active agent with varying concentrations. The variation threatment are consist of four treatments P1 (NaOCl 5.25%; 3 minutes), P2 (NaOCl 5.25%; 1,5 minutes), P3 (NaOCl 3%; 3 minutes), and P4 (NaOCl 3%; 1,5 minutes), which every treatments have one control medium and three repetition. TSA (Tryptic Soy Agar) Merck were applied for growing the isolated endophytic bacteria.

In all treatments, the leaf washed under running tap water to remove microorganisms and dust particles from the surface. 1 gram of *Cosmos caudatus* leaf were used for each treatment, these leafe than exposed to sterilization agent. First immersed in 70% ethanol for one minute, afterward exposed to the NaOCl with different concentrations and time exposure for every treatment, with few drops of Tween 80 were added to the solutions. The use of ethanol and NaOCl is to reduce contamination and by adding the Tween 80 is to enhance the coverage of the solution on the leaf. The sterilized leaf were then rinsed thoroughly with sterile distilled water 3 times each time to remove the disinfectant completely.

Using the stirilized leaf, it streak on the TSA medium as the control. The leaf than crushed and than suspended in 0.8% of physiological salt and it homogenized using vortex. 100 µL suspension of *C. caudatus* leaf with 10^{-1} serial dilution, is taken and inoculated on medium using spread methode, than it incubated overnight in temperature 37 ºC.

The result of each threatment and control than observed. If the control medium is setrile, means the sterilization process is successful and the growing bacteria on the repeatation medium is endopitic bacteria. The number of colonies than determined by counting the number of Colony Forming Units (CFU/mL). Data were subjected to analysis of variance (ANOVA) (degree of true= 95%), signification is 0.067 and means were compared by the Duncan's multiple range test at $p<0.05$ using the SPSS ver.20 (SPSS Inc., USA).

III. RESULTS AND DISCUSSION

The result (Table 1) showed that number of colony forming unit of endothypic bacteria decrease with an increase in NaOCl concentration and time of exposure. Results also show that at treatment P2 with 5,35% of NaOCl and 1,5 minutes time of exposure and P3 with 3% of NaOCl and 3 minutes time of exposure had best impact on viability of endothypic bacteria. At that treatment, 2,9 X 10^3 bacteria colony are formed.

Table.1: Results of various concentrations and time exposure of NaClO on number of colony forming unit

Treatment	NaOCl Consentration	Time Exposure	Number of CFU/ml
P1	5, 35%	3 minute	0 x 10^2
P2	5, 35 %	1,5 minute	2,9 X 10^3
P3	3 %	3 minute	2,9 X 10^3
P4	3 %	1,5 minute	2,8 X 10^3

Surface sterilization of *Cosmos caudatus* kunth. leaf using method I (P1) showed that with NaOCl 5,35% in 3 minute was effective to eliminate microorganisms on the leaf surface. NaOCl 5,35% in 3 minute possess antimicrobial activity and efficiently in eliminating the microorganism. At the same time, this treatment methode give impact to the number colony forming unit of endophytic bacteria. In this treatment the less number colony forming unit of endophytic bacteria can growth. 0 x 10^2 colony of endophytic bacteria are formed. It's mean

https://dx.doi.org/10.22161/ijaers.4.7.14 *ISSN: 2349-6495(P) | 2456-1908(O)*

that at this treatment NaOCl bactericide activity is stronger than the three other treatment.

In method III which has the same result with methode (P2), there are no contamination on the control tretament. This tereatment also perform good sufficient to eliminate surface microorganisms. Meanwhile the II and III methode show the best impact on colony forming unit of endophytic bacteria. 2,9 X 10^3 colony of bacteria can growth at the medium. Sodium hypochlorite in concentration combining with time esxposure give the different impact to the colony forming unit of endophytic bacteria. at high concentration with long time of exposure, potetianly to eliminate the microorgnism in the surface of *Cosmos caudatus* kunth leaf. But also as the bactericide to the endophytic bacteria.

Sodium hypochlorite NaOCl is a common use for surface sterilization. It is available as commercial bleach product and can be diluted to proper concentrations. Generally it use by immersing the target organ in this solution for 5-20 minutes. A balance between concentration and time should be verified for each type of plants. Sodium hypochlorite being known as effective disinfectant agent against to many bacteria. At the case that some bacteria can survive, is might be due to resistance towards sodium hypochlorite. The bactericidal activity of hypochlorite solution (Bleach) is the NaOCl (OCl^-) ion with the former being more active so that the disinfecting efficiency of chlorine is best in slightly acid hypochlorite solution (George, 1993 and Russel, 1996).

The results of Mahmoud and Al-Ani (2016), showed that treatment with sodium hypochlorite (NaOCl) had satisfactory results and Singh (2011) also showed that the best concentration of NaOCl is 3%, which can reduced bacterial contaminations up to 95%. Our results are in agreement with earlier studies on attempts using various sterilization methods. The sodium hypochlorite showed a high in reducing the contamination of bacteria because of its is very effective as disinfectant agent against many bacteria. Hypochlorite (OCl-) as a strong oxidant can denature by aggregating essential proteins of bacteria as previously described (Winter *et al*, 2008).

In our study the combination between contcetration and time exposure of NaOCl proved to be sterilizing agent that has different impact to the endophytic bacteria colony forming unit. In summary, 5, 35 % of NaOCl with 1,5 minute time exposure and 3 % of NaOCl with 3 minute of time exposure, show as the best sterilization methode to be very effective in eliminating surface microorganisms with high viability in endophytic bacteria.

IV. CONCLUSION

From the results obtained, it can be concluded that the effect of various concentrations and time exposure of NaClO on number of colony forming unit of endophytic bacteria of *Cosmos caudatus* kunth. Leaf The conclusion from this study is there was no significantly different effect of different concentration and time sterilization on the number of colony-forming units of isolated endophytic bacteria from *Cosmos caudatus* kunth. leaf.

REFERENCES

[1] Abas, F., Shaari, K., Lajis, N.H., Israf, D.A., dan Kalsom, Y.U., Antioxidative and Radical Scavenging Properties of the Constituents Isolated from Cosmos caudatus Kunth. Nat. Prod. Sciences 9 (4): 245-248.

[2] George EF. (1993). *Plant Propagation By Tissue Culture, Part 1. The Technology (2ndedn)*, exegetics, westbury, UK.

[3] Lotulung, P.D.N., Minarti dan Kardono, L.B.S. 2005. Penapisan Aktivitas Antibakteri, Antioksidan dan Toksisitas Terhadap Larva Udang *Artemia salina* Ekstrak Tumbuhan Asteraceae, Abstrak, Pusat Penelitian Kimia LIPI

[4] Mahmoud, S. N. and Al-Ani, N. K (2016). Effect of Different Sterilization Methods on Contamination and Viability of Nodal Segments of *Cestrum nocturnum* L. *International Journal of Research Studies in Biosciences (IJRSB)*, 4, p. 4-9.

[5] Mc.donnell G, Russell AD. (1999). Antiseptics and disinfectants: Activity, action, and resistance. *Clin Microbiol Rev* 12 p.147-179.

[6] Ministry of Health Republic of Indonesia. 2015. *Situation of Cancer*. Jakarta: Data Center and Information Ministry of Health Republic of Indonesia.

[7] Pebriana R.B., Wardhani B.W.K., Widayanti E., Wijayanti N.L.S., Wijayanti T.R., Riyanto S., dan Meiyanto E., 2008, Pengaruh Ekstrak Metanolik Daun Kenikir (*Cosmos caudatus* Kunth.) terhadap Pemacuan Apoptosis Sel Kanker Payudara, *Pharmacon*, 9(1), 21-26.

[8] Ren, W., Qiao, Z., Wang, H., Zhu, L., Zhang, L., 2003, Flavonoids: Promising Anticancer Agents, Medicinal Research Reviews, 23 (4), 519–534

[9] Singh, V., et al., Identification and prevention of bacterial contimination on explant used in plant tissue culture labs. *International Journal of Pharmacy and Pharmaceutical Sciences*, 3. p.160-163.

[10] Taraphdar, Amit, K., Madhumita, Roy, dan Bhattacharya, R.K., 2001, Natural products as inducers of apoptosis: Implication for cancer therapy and prevention, Current Science 80(11): 1391.

[11] Winter J, Ilbert M, Graf PC, Ozcelik D, Jakob U. (2008). Bleach activates a redox regulated chaperone by oxidative protein unfolding. Cell 135: 691-701.