Sensitivity of *Vibrio parahaemolyticus*, *V. vulnificus* and *V. harveyi* Against Chloroxylenol (4-Chloro-3,5-dimethylphenol, C8H9ClO) Antiseptic and Pine Oil Disinfectant

Subagiyo[1], Ervia Yudiati[1]*, Nuril Azhar[1], Rabia Alghazeer[2]

[1]*Marine Science Department, Faculty of Fisheries and Marine Science, Diponegoro University*
Jl. Prof. H. Soedarto, SH., Tembalang, Semarang, 50275, Indonesia
[2]*Department of Chemistry, Faculty of Sciences, University of Tripoli*
Tariq Sayyidi al Misri Road, Tripoli, Libya
Email: eyudiati@gmail.com

Abstract

Vibrio spp. genus is known as a marine indigeneous bacteria. Vibrio parahaemolyticus, V. vulnificus and V. harveyi are pathogenic Vibrio. This study aims to assess the sensitivity of three Vibrio species (V parahaemolyticus, V. vulnificus and V. harveyi) isolated from shrimp pond against two type of disinfectant with different active compound namely Chloroxylenol (4-Chloro-3,5-dimethylphenol, C8H9ClO) and pine oil. The assessment was done by Kirby-Bauer disk diffusion methods in Zobell agar media with two different concentration (10 and 100 ppm) and replicated in three times. Sensitivity of Vibrio spp. was analized based on the inhibition zone activity produced by disinfectant. Results showed that sensitivity of Vibrio spp. against disinfectant Chloroxylenol 4.8% at 100 ppm were higher than 10 ppm. The increment of V parahaemolyticus was 182 %, V. vulnificus was 47 % and V. harveyi was 43 %, respectively. Susceptibility of antiseptic with Chloroxylenol 4.8% at 100 ppm was arised to 152 % (V. parahaemolyticus), 43 % (V. vulnificus) and 31 % (V. harveyi) when compared to 2.5% pine oil disinfectant. It can be concluded that Chloroxylenol 4,8 % active compound and pine oil were able to inhibit the Vibrio spp. growth.

Keywords : *Vibrio spp.; antseptic; disinfectant; chloroxylenol; pine oil*

INTRODUCTION

Vibrio parahaemolyticus and Vibrio vulnificus are common bacteria from coastal and estuary zone. This bacteria are pathogenic to human (Huehn *et al*., 2014 ; Raszl *et al*., 2016). *Vibrio parahaemolyticus* infection is clinically sign by gastroenteritic and wound infection (Rezny *et al*., 2020), diarrhea, abdominal cramping, chills nausea, vomiting, and fever (Raszl *et al.*, 2016), and in more severe cases this can cause sepsis (Rezny *et al*., 2020).

V. parahaemolyticus' major virulence factor is Thermostable Direct Hemolysin (TDH) (Wang *et al*., 2015 ; Rezny *et al*., 2020) and TDH-related hemolysin (TRH) (Wang *et al*., 2015). On the other hand, *V. vulnificus* is an opportunistic pathogen to human (Gulig *et al*., 2005, Heng *et al*., 2017, Leng *et al*., 2019). *V. vulnificus* can cause primer sepsis, gastroenteretic and wound infection (Horseman & Surani., 2011; Al-Assafi *et al*., 2014 ; Raszl *et al*., 2016 ; Leng *et al*., 2019). The most important virulence factor of this bacteria are capsular polysaccharide (CPS), flagella and motility, acquisition of iron, hemolysin/cytolysin and metalloprotease as well as RtxA toxin (Gulig *et al*., 2005).

Vibrio harveyi is freely swim bacteria in tropical seawater and marine organism's gut microflora. This bacteria is a pathogenic to marine organisms, eventhogh, presumably is non pathogenic to human. Nevertheless, there are four articles reported the relation of *V. harveyi* infection to human (Pavia *et al*., 1989; Wilkins *et al*., 2008; Hundenborn *et al*., 2013; Gigia-Aguirre *et al*., 2017).

*) Corresponding author
www.ejournal2.undip.ac.id/index.php/jkt
Diterima/Received : 08-10-2020, Disetujui/Accepted : 20-10-2020
DOI: https://doi.org/10.14710/jkt.v23i3.9126

There are some hygiene procedure to counteract this disease problem. Hygiene is an early effort or action to keep and enhance the cleanliness and health by personal maintenance and environmental factors. The purpose if this activities is to avoid from the disease problem. So, therefore, the antiseptic practical become more popular and beneficial. Furthermore, people was also use disinfectant for environmental purpose. Chloroxylenol is a well known active compound for antiseptic. While, pine oil is an active compound for desinfectant.

Chloroxylenol is also known as p-chloro-m-xylenol, parachlorometaxylenol, 4-chloro-3,5-xylenol, 2-chloro-m-xylenol, 2-chloro-5-hydroxy-m-xylene, 2- chloro -5-hydroxy- 1, 3-di methyl benzene, 4-chloro-1-hydroxy-3,5-di methylbenzene, PCMX (Final Report on the Safety Assessment of p-Hydroxyanisole,1985). Due to the antibacterial and antifungi activity, Chloroxylenol is commonly used as disinfectant, preservative, topical antiseptic (Final Report on the Safety Assessment of p-Hydroxyanisole.,1985 ; Moore & Payne., 2013) and disinfectant (Moore & Payne 2013). To improve the PCMX solubility, people used to diluted with soap and combined with terpineol (Moore & Payne 2013). The assessment on antiseptic activity of chloroxylenol has been proven for *Staphylococcus aureus, Klebsiella species, Salmonella typhi, Shigella dysenteriae*, (Saha *et al*., 2009), *Staphylococcus aureus*, *Micrococcus* spp., *Staphylococcus* spp, *Bacillus* spp., *Pseudomonas aeruginosa* (Hassan & El Bagoury, 2018 ; Al-Talib *et al*., 2019), methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii, Klebsiella species* (Al-Talib *et al*., 2019). Moreover, this antiseptic is able to decrease the total bacterial count for hand sanitizer (Riza *et al*., 2019).

Pine oil is an active compound for disinfectant purposes. Some researchers show that pinus oil have an antimicrobial activity. The essensial pinus inhibited *Staphylococcus aureus* growth (Fit *et al*., 2009 ; Joshua *et al*., 2014; Leandro *et al*., 2014; Raho, 2014), *Escherichia coli* (Raho, 2014), *Staphylococcus epidermidis, Staphylococcus aureus, Enterococcus faecium, Staphylococcus capitis, Enterococcus faecalis, Staphylococcus haemolyticus*, and *Klebsiella pneumonia* (Leandro *et al*., 2014).

Up to now, the information concerning the activity of Chloroxylenol and pine oil against *Vibrio* spp., especially *V. parahaemolyticus, V. vulnificus dan V. harveyi* is unavailable. This research is providing this information to find out the sensitivity of these three Vibrio species against chloroxylenol. This data is useful as a basic infromation of chloroxylenol and pine oil application as an antiseptic as well as disinfectant to counter *Vibrio* spp.

MATERIALS AND METHODS

Vibrio spp. isolates

Vibrio parahaemolyticus, V. vulnificus and *V. harveyi* was obtained from microbia collection of Biology Laboratory, Faculty of Fisheries and Marine Science, Diponegoro University. Sub-culture was applied, prior before used. Sub-culture was done in order to cultivate the microorganisms by inoculating one ose from stock bacteria to 5 mL nutrient broth media. This was then incubated for 24 hrs at 37 ^{o}C.

Antiseptic material

Antiseptic used in this research was commercial antiseptic with Chloroxylenol 4.8% w/v active compound and commercial disinfectant with pine oil 2,5% active compound.

Antiseptic Dilution

The concentration of antiseptic and disinfectant used in this trial was 10 and 100 ppm. Ten ppm concentration was prepared by diluting 0.01 mL to one L of aquadest. While antiseptic was prepared by diluting 0.1 mL antiseptic to the similar volume of aquadest.

Turbidity standard for innoculum preparation

Standard density of bacterial test for sensitivity assessment in this research was used $BaSO_4$ turbidity standard which is optically

equal to 0,5 McFarland. Standard $BaSO_4$ 0.5 McFarland was prepared by adding 0.5 ml aliquot 0,048 mol / L $BaCl_2$ (1,175% w / v $BaCl_2$. $2H_2O$) into 99,5 ml 0,18 mol / L H_2SO4 (1% v / v), and stirred well. The standard density was verified with 1 cm spectrophotometer light with suitable cuvette to determine the absorbance. The standar 0.5 McFarland (625 nm) has to be around 0.008-0.13. The standard was then placed in a covered tightly tube, kept in room temperature without any light. The barium sulfat standard was stirred well in the vortex before used and carefully checked to ensure the homogeneity (Hudzicki, 2009).

Sensitivity Test of *Vibrio* spp Against Antiseptic

Sensitivity test of *Vibrio* spp. against antiseptic was done by Kirby-Bauer methods (Hudzicki, 2009). Subcultured *Vibrio* spp. was inoculated to nutrient broth medium, incubated in 24 hrs at 37°C. Bacterial density was adjusted to the standar 0,5 McFarland (1-2 x 10^8 CFU). Sterile cotton was immerged into subcultured bacteria then spreaded gently at the surface of NA media in petridish. Leave it for 5 minutes, then sterile paper disk was placed at the surface of NA media-inoculated with *Vibrio* spp. Similar procedure was apllied to the pine oil disinfectant. Incubation was administered at 24 hrs in room temperature. Each treatment was replicated in three times. Sensitivity of *Vibrio* spp. against antiseptic and disinfectant material was counted based on the production of inhibition zone.

Principles interpretation of inhibition zone for bacterial sensitivity against antiseptic was based on Wanja *et al*., (2020). Range of 0 – 5 mm from the periphere of petridish was categorized as weak inhibition. Range of 6 – 9 mm is categorized as moderate inhibition. Range of 10 - 14 mm is cathegorized as strong inhibition, whereas >15 mm is cathegorized as very strong inhibition.

RESULTS AND DISCUSSION

Chloroxylenol is a halophenol (Bednarek *et al.,* 2020) which has the antimicrobial activity (USEPA, 1994). This due to the phenolic characters which interfere the microbial membrane (McDonnell & Russell, 1999), damaging the microbe cell wall (Bednarek *et al.*, 2020), inactivated the cellular enzime (USEPA, 1994 ; Bednarek *et al.,* 2020) and also denatured the proteins (USEPA, 1994).

The explanation of mechanism is, the phenolic antiseptic of chloroxylenol contains –OH (hydroxyl) functional group. This hydroxyl group will bond to particular protein in the bacterial cell membrane resulting bacterial breakdown. Since there is a leakage of bacterial plasma cell, this will push chloroxylenol to enter into bacerial cell and continue to bond more proteins and enzymes. So, therefore, this will be inactivated the cell function. At highly chloroxylenol concentration, targetted bacterial protein and nucleic acid become coagulated and all the function has stopped. Finally, the rapid cell mortality was occurred (https://www.drugbank.ca/drugs/DB11121).

This present research was conducted by diluting the commercial antiseptic to 10 and 100 ppm (v/v). The stock concentration is 4.8 % (w/v). This can be calculated, that the chloroxylenol active compound from 10 ppm was 0,00048 mg/100 mL. While 100 ppm contained 0,0048 mg/100 mL. Results from Figure 1A and Table 1 showed that 10 ppm poduced diameter inhibition zone of 8.53 mm for *V. parahaemolyticus*, 16.52 mm for *V. vulnificus* (Figure 1A) and 16.10 mm for *V. harveyi (*Table 1*)*, respectively. Based on diameter inhibition zone produced, this showed that *V. vulnificus* dan *V. harveyi* were more sensitive to chloroxylenol, even at low concentration. Eventhough, at higher concentration (100 ppm), it showed the similar inhibition zone (Table 1) which is 24.07, 24.33 and 23.07 mm towards *V. parahaemolyticus* (Figure 1B)*, V. vulnificus* (Figure 1C) and *V. harveyi* (Figure 1D), respectively. According to inhibition zones categorized by Wanja *et al*., (2020), the 10 ppm was categorized as weak inbition, while 100 ppm was cateorized as moderate inhibition (Table 1). In fact, this present study was also tested at the 1000 ppm though the results of inhibition zone was too wide and spreaded all over the petridish. This results proofed that chloroxylenol have the powerful inhibition activity against *Vibrio* spp. In terms

Table 1. Diameter of Vibrio spp. Inhibition zone (mm) against antiseptic and disinfectant

Active compound	Concentration (ppm)	Bacteria	Diameter of inhibition zone (mm)	Percentage enhancement (%)	Inhibition zone of paper disc peripheral
antiseptic (chloroxylenol)	10	*V. parahaemolyticus*	8.53±1.81		1.27(R)
		V. vulnificus	16.52±1.51		5.26(R)
		V. harveyi	16.10±1.89		5.05(R)
	100	*V. parahaemolyticus*	24.07±1.89	182	9.04(R)
		V. vulnificus	24.33±1.21	47	9.17(R)
		V. harveyi	23.07±0.85	43	8.54(R)
disinfectant (pine oil)	10	*V. parahaemolyticus*	6.175±0.29		0.09(R)
		V. vulnificus	6.95±0.47		0.48(R)
		V. harveyi	13.43±2.98		3.72(R)
	100	*V. parahaemolyticus*	9.52±0.69	54	1.76(R)
		V. vulnificus	17.02±2.61	144	5.51(R)
		V. harveyi	17.5±1.96	30	5.75(R)

Denoted : value above is average and standart deviation (n=3). Percentage of inhibition zone enhancement from 10 to 100 ppm was counted based on the measurements of inhibition zone produced from the peripheral of paper disk outward. R : weak inhibition, S : moderati inhibition

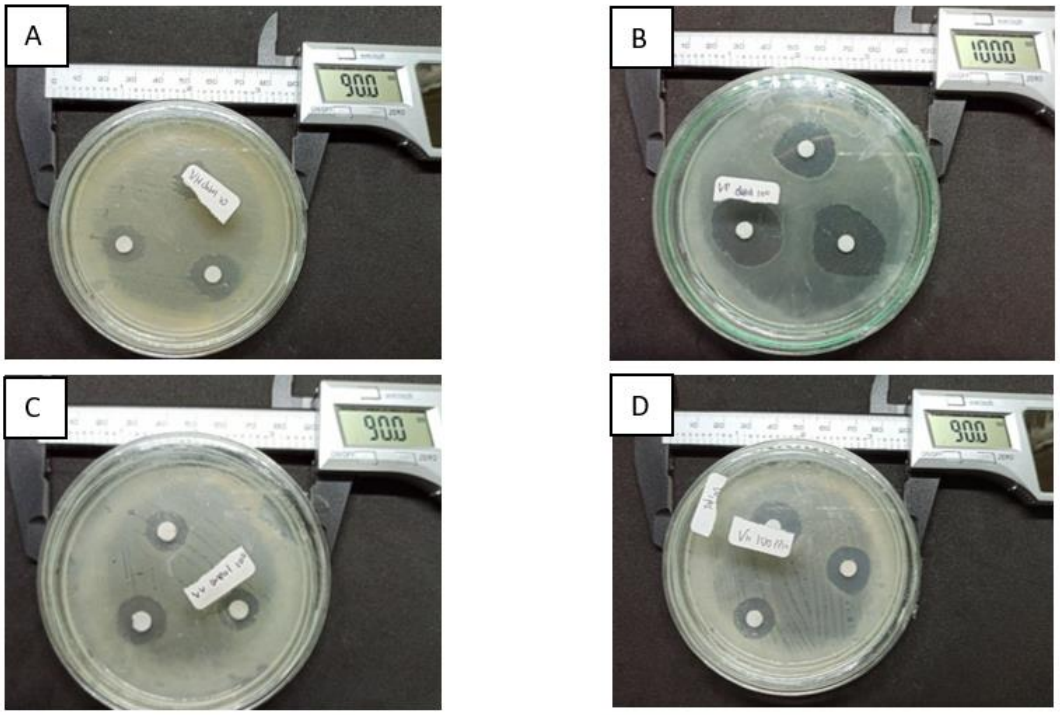

Figure 1. Paper disc inhibition zone A). chloroxylenol active compound at 10 ppm (*V. harveyi*); B) pine oil active compound at 100 ppm (*V. harveyi*); C) pine oil active compound at 100 ppm (*V. vulnificus*); D) chloroxylenol active compound at 100 ppm (*V. parahaemolyticus*)

of pine oil, people used to treat pine oil for disinfection agent. According to this results, pine oil produced less inhibition zone, compared to chloroxylenol (Table 1).

Based on the power of disinfectant categorized by Wanja *et al*., (2020), it can be clearly shown that pine oil have the less inhibition one against *Vibrio* spp. at 100 ppm dilution. The major componen of pine oil is a-terpineol and terpinolene (Tadtong *et al.,* 2015). Oyedemi *et al*. (2009) confirmed that a-terpineol gave the strong effect to the leakage of gram negative and gram positive

bacteria which leads to the disturbance of the outer membrane. Furthermore, a-terpineol was also damaged the cell wall which will stimulate the lipid leakage.

This is confirming that pine oil has the mechanism as disinfectant by damaging the membrane and cell wall against gram positive and gram negative bacteria. Li *et al.*, (2014) reported some research concerning to the application of a-terpineol 0.78 µl/mL against bacteria *Escherichia coli* (CMCC (B) 44102). Their research showed some evidence that the size cell was reduced and became irregular, the wall and cell membrane were broken, cytoplasmic nucleus was reduced and nucleus area was aggregated.

CONCLUSION

This research confirmed that *V. parahaemolyticus, V. vulnificus* and *V. harveyi* were sensitive against antiseptic chloroxylenol active compound. This has proven by the production of inhibition zone at low concenctration (10 ppm). The sensitivity of *Vibrio* spp. against antiseptic chloroxylenol was even higher compared to disinfectant contains pine oil antiseptic. Based on the results above, it can be concluded that antiseptic contains chloroxylenol active compound can be applicated to combat or inhibit *Vibrio* spp. growth. Moreover, disinfectant contains pine oil active compound is suitable for the application of Vibrio spp. disinfection.

ACKNOWLEDGEMENT

The authors would like to address a high appreciation to the Faculty of Fisheries and Marine Science, Diponegoro University for the financial funds. This research is completely granted through non APBN scheme with contract no: 172/UN7.5.10/PM/2019.

REFERENCES

Adams, J., Gibson, K.E., Martin, E.M., Almeida, G., Ricke, S.C. & Frederick, N. 2014. Characterization and variation of essential oil from Pinus taeda and antimicrobial effects against antibiotic-resistant and susceptible *Staphylococcus aureus*. *Forest Prod. J.*, 64 (5-6). doi: 10.13073/ FPJ-D-14-00018

Al-Assafi, M.M.K., Abd Mutalib, S., Ghani, M.A. & Aldulaimi, M. 2014. A Review of Important Virulence Factors of Vibrio vulnificus. *Curr. Res. J. Biol. Sci.,* 6(2):76-88, doi:10.19026/crjbs.6.5502

Al-Talib, H., Alkhateeb, A., Syahrizal, A., Ruzuki, A., Zulkifli, N.F., Hamizi, S., Muhammad, N.S. & Karim, A.F.A. 2019. Effectiveness of commonly used antiseptics on bacteria causing nosocomial infections in tertiary hospital in Malaysia, *Afr. J. Microbiol. Res.,* 13(10):188-194. doi: 10.5897/AJMR2019.9058

Bednarek R. S., Nassereddin, A. & Ramsey, M.L. 2020. Skin (Integument) Antiseptics. [Updated 2020 Jun 3]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK 507853/

EL Mahmood, A.M. & Doughari, J.H. 2008. Effect of Dettoll on viability of some microorganisms associated with nosocomial infections, *Afr. J. Microbiol. Res.,* 7(10):1554-1562. doi: 10.5897/AJB08.052

Final Report on the Safety Assessment of p-Hydroxyanisole. 1985. *J. Am. Coll. Toxicol.*. *4*(5):31–63. doi: 10.3109/10915818509078686

Fit, I.N., Rapuntean, G., Rapuntean, S., Chirila, F., & Nadas, G.C. 2009, Antibacterial Effect of Essential Vegetal Extracts on Staphylococcus aureus Compared to Antibiotics, *Not. Bot. Hort. Agrobot. Cluj* 37(2):117-123 DOI: 10.15835/nbha3723183

Gigia-Aguirre, L.D., Sánchez-Yebra-Romera, W., García-Muñoz, S. &. Rodríguez-Maresca, M. 2017, First description of wound infection with Vibrio harveyi in Spain, *New Microbes New Infect.* 19:15–16. doi: 10.1016/j.nmni.2017.05.004

Gulig, P.A., Bourdage, K.L. & Starks, A.M. 2005, Molecular Pathogenesis of Vibrio vulnificus, *J. Microbiol.,* 43(S):118-131. PMID: 15765065.

Hassan, K. & El Bagoury, M., 2018, Antimicrobial Activity Of Some Biocides Against Microorganisms Isolated From A Shared Student Kitchen, Rasayan *J. Chem.,* 11(1):238-244,doi: 10.7324/RJC.2018.1112019

Heng S.P., Letchumanan, V., Deng, C.Y., Ab

Mutalib, N.S., Khan, T.M., Chuah, L.H., Chan, K.G., Goh, B.H., Pusparajah, P. & Lee, L.H. 2017. *Vibrio vulnificus*: An Environmental and Clinical Burden. *Front. Microbiol*. 8:997. doi: 10.3389/fmicb.2017.00997

Horseman M.A. & Surani, S. 2011. A comprehensive review of Vibrio vulnificus: an important cause of severe sepsis and skin and soft-tissue infection, *Int. J. Infect. Dis.* 15:157–166. doi: 10.1016/j.ijid.2010.11.003

http://www.fao.org/3/ca6028en/ca6028en.pdf

https://www.drugbank.ca/drugs/DB11121).

Hudzicki, J., 2009, Kirby-bauer disk diffusion susceptibility test protocol, American Society for Microbiology.

Huehn, S., Eichhorn, C., Urmersbach, S., Breidenbach, J., Bechlars, S., Bier, N., Alter, T., Bartelt, E., Frank, C., Oberheitmann, B. & Gunzer, F., 2014, Pathogenic vibrios in environmental, seafood and clinical sources in Germany. *Int. J. Med. Microbiol.*, *304*(7): 843–850. doi: 10.1016/j.ijmm.2014.07.010

Hundenborn J., Thurig, S., Kommerell, M., Haag, H. & Nolte, O. 2013. Severe wound infection with Photobacterium damselae ssp. damselae and Vibrio harveyi, following a laceration injury in marine environment: a case report and review of the literature. *Case Rep. Med.* 2013:610632. doi: 10.1155/2013/610632

Jain, A., Jain, R. & Jain, S. 2020, Basic Techniques in Biochemistry, Microbiology and Molecular Biology_ Principles and Techniques, Humana Press, Springer Protocols Handbooks, doi: 10.1007/978-1-4939-9861-6

Leandro, L.F., O.Cardoso, M.J., Silva, S.D.C., Souza, M.G.M., Veneziani, R.C.S., Ambrosio, S.R. & Martins, C.H.G. 2014, Antibacterial activity of Pinus elliottii and its major compound, dehydroabietic acid, against multidrug-resistant strains, *J. Med. Microbiol*., 63:1649–1653. DOI: 10.1099/jmm.0.081711-0

Leng, F., Lin, S., Wu, W., Zhang, J., Song, J. & Zhong, M., 2019. Epidemiology, pathogenetic mechanism, clinical characteristics, and treatment of Vibrio vulnificus infection: a case report and literature review, *Eur. J. Clin. Microbiol. Infect. Dis.,* 38:1999–2004, doi: 10.1007/s10096-019-03629-5

Li, L., Shi, C., Yin, Z., Jia, R., Peng, L., Kang, S., & Li, Z. 2014, Antibacterial activity of a-terpineol may induce morphostructural alterations in *Escherichia coli*. *Braz. J. Microbiol.* 45(4):1409-13, doi: 10.1590/S1517-8382201 4000400035

Mcdonnell, G. & Russell, A.D., 1999, Antiseptics And Disinfectants: Activity, Action, And Resistance, Clinical Microbiol. Rev., 2(1):147–179. doi: 10.1128/CMR.12.1.147

Moore, S., & Payne, D.N., 2013. Types of Antimicrobial Agents, In Principles and Practice of Disinfection, Preservation & Sterilization, 5th Edition, Fraise, A. P., J.Y. Maillard, S. A. & Sattar (ed), A John Wiley & Sons, Ltd., Publication. doi: 10.1002/9780470755884.ch2

Oyedemi, S.O., Okoh, A.I., Mabinya, L.V., Pirochenva, G. & Afolayan, A.J. 2009, The proposed mechanism of bactericidal action of eugenol, -terpineol and γ-terpinene against Listeria monocytogenes, *Streptococcus pyogenes*, *Proteus vulgaris* and *Escherichia coli*, *Afr. J. Microbiol. Biotechnol.*, 8(7):1280-1286. doi: 10.4314/ajb.v8i7.60106

Pavía A.T., Bryan, J.A., Maher, K.L., Hester, T.R. & Farmer, J.J. 1989. *Vibrio carchariae* infection after a shark bite. *Ann. Intern. Med.* 111:85–6. doi: 10.7326/0003-4819-111-1-85

Raho, G.B., 2014. Antibacterial potential of essential oils of the needles of Pinus halepensis against *Staphylococcus aureus* and *Escherichia coli*. *J. Coast. Life Med.*, 2(8):651-655., doi: 10.12980/JCLM.2.2014APJTD-2014-0041

Raszl, S.M., Froelich, B.A., Vieira, C.R., Blackwood, A.D., & Noble, R.T. 2016. *Vibrio parahaemolyticus* and *Vibrio vulnificus* in South America: water, seafood and human infections. *J. Applied Microbiol.*, *121*(5):1201–1222. doi: 10.1111/jam.13246

Rezny, B.R. & Evans, D.S. 2020. *Vibrio Parahaemolyticus*. [Updated 2020 Jul 3]. In: StatPearls [Internet]. Treasure Island

(FL): StatPearls Publishing; 2020 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459164/

Riza, A., Isnandar, I., Syaflida, R. & Jasmine, J., 2019. Comparison of chloroxylenol 4.8% and povidone iodine 7.5% on total bacteria count post WHO routine hand washing on clinical students at the Department of Oral Surgery, Faculty of Dentistry, Universitas Sumatera Utara March-May 2018. J. Dentomaxillofacial Sci. 4(3):142-144. DOI: 10.15562/jdmfs.v4i3.796

Saha. A.K., Haque, M.F., Karmaker, S. & Mohanta, M.K. 2009. Antibacterial Effects of Some Antiseptics and Disinfectants, *J. Life Earth Sci.,* 3-4:19-21, doi: 10.3329/jles.v3i0.7440

Tadtonga, S., Kamkaenb, N., Watthanachaiy-ingcharoena, R. & Ruangrungsi, N. 2015. Chemical Components of Four Essential Oils in Aromatherapy Recipe, *Nat. Prod. Comm*. 10(6):1091–1092. doi: 10.1177/1934578X15 01000673

USEPA (United States Environmental Protection Agency) 1994, Reregistration Eligibility Decision (RED) Chloroxylenol, Washington, D.C. 20460

Wang R., Zhong, Y., Gu, X., Jun, Y., Abdullah, S. & Wang, S. 2015. The pathogenesis, detection, and prevention of *Vibrio parahaemolyticus. Front. Microbiol.*, 6:144. doi: 10.3389/fmicb.2015.00144

Wanja, D.W., Mbuthia, P.G., Waruiru, R.M., Bebora, L.C., Ngowi, H.A. & Nyaga, P.N. 2020. Antibiotic and Disinfectant Susceptibility Patterns of Bacteria Isolated from Farmed Fish in Kirinyaga County, Kenya. *Int. J. Microbiol*., 2020(8897338):8 pages, doi: 10.1155/2020/8897338

Wilkins S., Millar, M., Hemsworth, S., Johnson, G., Warwick, S. & Pizer, B., 2008, *Vibrio harveyisepsis* in a child with cancer. *Pediatr Blood Cancer*, 50:891–2. doi: 10.1002/pbc.21356