Research Journal of Life Science
AUGUST-2019 Volume 6 NO. 2 (72-82)
journal homepage: www.rjls.ub.ac.id

Minimum Inhibitory Concentration of Marine Microalgae *Dunaliella salina* on Fish Pathogenic Bacteria *Edwardsiella tarda*

Rusmawanto, Arief Prajitno, Ating Yuniarti

Department of Aquaculture, Faculty of Fisheries and Marine Science, University of Brawijaya.

Email address: rusmawanto49@gmail.com

KEYWORDS

Antimicrobial
Dunaliella salina
Edwardsiella tarda
Minimum inhibitory Concentration

Abstract *Dunaliella salina* is a type of marine microalgae. The objective of this research were investigated the effect of *D. salina* to inhibit the growth of *E. tarda*. The method used in this study was examined antibacterial activity of *D. salina* using disc diffusion and co culture test. The secondary metabolite compound in *D. salina* was tested using phytochemical screening and FTIR methods. The results obtained *D. salina* extracted using n-hexane showed the best activity for inhibiting the growth of *E. tarda*. Minimum concentration of 100 mg.L^{-1} crude extract can inhibit *E. tarda* with a total of bacterial colonies 137x10^{11} CFU.mL^{-1}. The phytochemical test results showed that *D. salina* extract using n-hexane contained flavonoids, saponins, alkaloids, terpenoids and phenols. The FTIR analysis showed phenol and carotene compounds are one of the secondary metabolites that can be used as antibacterial. It can be concluded that the extract has strong antibacterial activity against *E. tarda* and potentially as antibacterial in aquaculture.

Introduction

One of the techniques in aquaculture to pursue the production target by implementing an intensive system. The aquaculture production sustainability influenced by pathogenic microorganisms. This condition is positively correlated with the more intensive cultivation systems developed (Cao *et al*., 2007). The application of this intensive aquaculture system can also cause fish susceptible to bacterial and viral diseases (Labh and Shakya, 2014; Mehana *et al*., 2014).

In the aquaculture system, bacteria are considered the main cause of infection in intensive aquaculture system of fish or shrimp in the world (Jin *et al.,* 2012; Austin and Austin, 2016). One species of this bacterium is *Edwardsiella tarda*. This infection of *E. tarda* causes disease attacks, commonly called Edwardsiellosis (Firma *et al.,* 2012; Li *et al.*, 2015). The prevalence of deaths due to *E. tarda* infection reaches 100% in common carp (*Charassius auratus*), celebes rainbow (*Telmatherina celebensis*) and carp types *Catla catla* (Narwiyani and Kurniasih, 2011; Devi *et al.*, 2016). Not only that, the infection of *E. tarda* can also cause economic losses. Many research showed that economic losses due to E. *tarda* infection on Japanese flounder (*Paralichthys olivaceus*) reached 487.9 billion Korean Won in 2010 years (Park *et al*., 2012; Syafitrianto *et al*., 2016).

Antibiotics commonly used as treatment to control *E. tarda* infection (Kholil *et al.*, 2015). However, the use of antibiotics can cause bacteria to be resistant to some antibiotics that has been used (Kadlec *et al.,* 2011; Kathleen *et al.,* 2016; Qiao *et al.*, 2018). Several studies have been conducted to determine the resistance of *E. tarda* due to the use of antibiotics. Among them mentioned that *E. tarda* is resistant to antibiotics colistin, tetracycline and trimethoprim or sulfamethoxazole (Shin *et al.,* 2017). Furthermore, antibiotics from the types

How to cite this article: Rusmawanto, Prajitno, A., Yuniarti, A. (2019). Minimum Inhibitory Concentration of Marine Microalgae *Dunaliella salina* on Fish Pathogenic Bacteria *Edwardsiella tarda.* Research Journal of Life Science 6 (2), 72-82.
https://doi.org/10.21776/ub.rjls.2019.006.02.1

of nalidixic acid, streptomycin, levofloxacin and amoxycillin also cause resistance to *E. tarda* bacteria (Ogbonne *et al.*, 2018).

Resistant bacteria will transfer genes to another bacteria by conjugation, transformation and transduction, which will produce bacteria that are resistant to certain antibiotics (Bbosa *et al.,* 2014). The resistance that occurs will also cause bacteria to be resistant to the drugs given, so the death of fish will increase. Liu *et al.* (2017) added another effect of the use of chemicals or antibiotics is that residues can accumulate in the fish's body and endanger humans who consume it. Therefore, the other alternative materials are needed as candidates for antibiotic substitutes from natural ingredients, which is microalgae.

In marine resources, microalgae are considered as the actual producers of some highly bioactive macromolecules, including carotenoids, long-chain polyunsaturated fatty acids, proteins, chlorophylls, vitamins, and unique pigments (Pasquet *et al.,* 2011). Some secondary metabolites derived from marine microalgae have the potency to be the new material for pharmacy (Ely *et al.*, 2004). Marine microalgae constitute attractive sources of novel and active metabolites, comprising proteins, enzymes, pigments and polyunsaturated fatty acids (PUFA) that could be exploited in pharmaceutical, food, feed and cosmetic industries (Mendes *et al.*, 2003; Cardozo *et al*., 2007; Surendhiran *et al.*, 2014).

Compounds with pharmaceutical characteristics, as antioxidative, anti-inflammatory, antimicrobial or antitumoral properties, has been identified. Some of them has been in the clinical trial state (Guedes *et al.*, 2011). Antimicrobial activities are among the most researched features in natural extracts. They have been attributed to different compounds, including, indoles, terpene derivatives, acetogenins, phenols, fatty acids and hydrocarbons (Bhakuni and Rawat, 2005; Keskin *et al.*, 2010).

In recent years, microalgae in aquaculture has been further developed where its use has the potential to inhibit bacterial growth (Falaise *et al*., 2016; Kokou *et al*., 2012). Some types of microalgae used to inhibit bacterial growth include *Scenedesmus obliquus* (Guedes *et al*., 2011), methanol extract *Chlorella vulgaris* (Syed *et al*., 2015), *Isochrysis galbana* and *Pavlova lutheri* microalgae (Bashir *et al*., 2018). The other microalgae can be used to inhibit bacterial growth in aquaculture environments are marine microalgae *D. salina* (Jafari *et al*., 2018).

D. salina has various important components such as, flavonoids and phenolic and the highest content is carotenoids (Lamers *et al*., 2010; Cakmak *et al*., 2014). This content can be used as an antibacterial candidate (Jafari *et al*., 2018). The other studies also state that the high content of protein, vitamins and minerals and the active compounds of chlorophyll α and β-carotene in *D. salina* can be used for fish health management as antiviral, antioxidant and antimicrobial (Bhat and Madyastha, 2000). The bacterial species tested challenge is a type of pathogenic bacteria that easily invade the freshwater, brackish water and brine water fish. So, by testing in vitro results of an extract of *D. salina*, we get pertinent information about power extracts of *D. salina* against *E*. *tarda*.

Materials and methods

This research was conducted in December to January 2019 at the Laboratory of Fish Health Disease (Faculty of Fisheries and Marine Sciences, University of Brawijaya), Laboratory of Materia Medika Batu, and Organic Chemistry Laboratory (Faculty of Science and Technology, State Islamic University of Malang). The laboratory equipment used in this study, is a set of rotary evaporator, phytochemical analysis and FTIR analysis. The materials used in this study, marine microalgae *D. salina* were

E-ISSN 2355-9926

collected from Brackishwater Aquaculture Center (BBAP) Situbondo, East Java, Indonesia.

Microalgae Extraction

Extraction process was performed based on the methods of Jafari *et al*. (2018), with some modification. *D. salina* powder (200 g) macerated with a ratio of 1:5 (w/v), soaked in 1000 mL of solvent for 1 days using hexane and ethyl acetate. After 24 hours on a closed erlenmeyer flask filtered through Whattman filter paper No. 42 to obtain a clear filtrate. The filtrate were evaporated and dried at 40°C with a rotation speed of 80 rpm under reduced pressure using rotatory vacum evaporator. The extract yields were weighted, stored in a small bottle in fridge at 5°C.

Bacterial preparation

The isolate of *E. tarda* was originated from Jepara Brackishwater Aquaculture Center. These bacteria were kept in *Trypticase Soy Agar* (TSA) media at 4°C and sub-cultured *Trypticase Soy Broth* (TSB) in overnight before use.

Antimicrobial assay

The antimicrobial activities of the crude extract were assayed against bacteria. The extract was weighed according to the dosage used and dissolved in 10% dimethyl sulfoxide (DMSO) at an initial concentration of 1000 $mg.L^{-1}$, 500 $mg.L^{-1}$, 100 $mg.L^{-1}$, 10 $mg.L^{-1}$, 1 $mg.L^{-1}$ and used a positive and negative control. Positive control used 5 $mg.L^{-1}$ synthetic antibacterial (*Chloramphenicol*) and negative control is only given by *E. tarda* bacteria.

Antimicrobial assay was carried out using the disc diffusion method and co-culture method. In the disc test, Muller Hinton Agar (MHA) in plates were inoculated with 0.1 mL each bacterium suspension overnight culture is adjusted to a 0.5 McFarland turbidity standard (10^7 $CFU.mL^{-1}$) and uniformly spread out. The plates were incubated at 37°C for 24 and 48 hours after the incubation period the inhibition zone was around the discs were measured and recorded.

In the co-culture test, Muller Hinton Broth (MHB) liquid media was given a bacterial inoculum of 10^7 $CFU.L^{-1}$ in a test tube, then extracted a *D. salina* crude extract by dilution method. Each treatment concentration was incubated for 24 hours at 37°C. The absorbance value of the media was measured by spectrophotometer. The bacterial subculture was then carried out from each treatment, both growing and not. On the subculture, each treatment was grown on Trypticase Soy Agar (TSA) media, then incubated for 24 hours at 37°C to determine the inhibitory power of bacterial growth.

Phytochemical analysis

The phytochemical analysis was carried out in accordance with (Evans, 2002) method. It was aimed to observe the active compound in crude extract. Compounds were analyzed include: flavonoids, alkaloids, phenolic, steroids, tannins and saponins.

FTIR (Fourier Transform Infrared Spectroscopy) analysis

As many as 1 mg of *D. salina* crude extract was crushed with 100 mg KBr (Potassium Bromide) homogeneously. Then, it measured by infrared ray absorption at a wavelength of 4000-400 cm^{-1} to determine the functional group of *D. salina* crude extracts.

Results and discussions

Antimicrobial Test

The results of the antimicrobial assay of the crude extract of *D. salina* against *E. tarda,* it can be seen in Table 1.

E-ISSN 2355-9926

Table 1. The results of disc test *D. salina* against *E. tarda*

Concentration Extract ($mg.L^{-1}$)	Clear Zone Diameter (mm)		Classification of Bacterial Inhibitory Responses
	24 hours	48 hours	
(K-)	0±0.0	0±0.0	Weak
1	0±0.0	0±0.0	Weak
10	0±0.0	0±0.0	Weak
100	6.92±0.40	7.72±0.19	Medium
500	16.71±1.09	16.14±0.80	Strong
1000	20.33±0.14	20.07±0.19	Very Strong
(K+)	25.24±0.09	25.64±0.68	Very Strong

Note: Classification of clear zone diameter, weak ≤ 5 mm, medium 5-10 mm, strong 10-20 mm, very strong ≥ 20 mm (Davis and Stout, 1971).

The largest inhibition zone is 1000 $mg.L^{-1}$ (20.33±0.14 mm) indicating that the inhibitory response concentration of bacteria is very strong. This means that the higher of *D. salina* crude extract, the more the inhibitory activity against *E. tarda*. The smallest or largest of the inhibition zone is also influenced by several factors including the toxicity of the test material, the diffusion ability of the test material in the media, the interaction between media components, and the in vitro microenvironment conditions (Candrasari *et al.,* 2012).

The follow-up test was carried out by the co-culture method. The difference results of co-culture test of *D. salina* using n-hexane and ethyl acetate solvent have obtained the data presented in Table 2.

Table 2. The result of co-culture test

Concentration Extract ($mg.L^{-1}$)	N-hexane		Ethyl Acetate	
	Absorbance (Å)	Colony Forming Unit ($CFU.mL^{-1}$)	Absorbance (Å)	Colony Forming Unit ($CFU.mL^{-1}$)
Control (-)	1.724	238×10^{20}	1.766	243×10^{21}
1	1.325	227×10^{20}	1.426	231×10^{21}
10	1.246	190×10^{19}	1.368	226×10^{21}
100	0.098	137×10^{11}	1.133	212×10^{18}
500	0.124	110×10^{9}	0.245	155×10^{11}
1000	0.162	63×10^{7}	0.362	127×10^{10}
Control (+)	0.065	0	0.068	0

Note: Positive control using chloramphenicol 5 $mg.L^{-1}$, negative control only bacteria.

Based on Table 2. the data show that minimum concentration of *D. salina* extract can be inhibit *E. tarda* bacterial growth is 100 $mg.L^{-1}$ in n-hexane solvents while in ethyl acetate at a dose of 500 $mg.L^{-1}$. This difference can be seen from the colony forming unit value of the extract and the absorbance value of the solution after being tested using a spectrophotometer. The best co-culture value was obtained from a minimum dose of extract whose absorbance value was close to positive control.

E-ISSN 2355-9926

Phytochemical screening

The results of phytochemical analysis observations showed the difference results *D. salina* extracted using 2 solvents, were obtained in Table 3.

Table 3. Phytochemical analysis for the hexane and ethyl acetate extract of *D. salina*

Metabolites	Characteristic	Result	
		Hexane	Ethyl acetate
Alkaloid	Orange Color Reaction (Dragendrof)	+	+
	White Sediment Reaction (Meyer)	+	+
	Brown Sediment Reaction (Bouchardat)	+	+
Flavonoid	Brick Red Color Reaction	+	+
Saponin	Stable Foam	+	+
Tannin	Green-Blackish Color Reaction	-	-
Terpenoid	Brownish Color Reaction	+	-
Phenol	Green-Blackish Color Reaction	+	+

Note: Positive (+) detected and negative (-) not detected.

The results of phytochemical screening have found secondary metabolite. Preliminary qualitative phytochemical analysis made for the crude extract of D. *salina* revealed the presence of alkaloids, flavonoids, saponins, tannins, terpenoids and phenols. They are reported to have many biological and therapeutic properties (Prasad *et al*., 2012; Anusha and Bai, 2017) so this microalgae is expected to have many medicinal uses. From this table we can declare that tannins not detected with n-hexane solvent. While, tannins and terpenoids not detected with ethyl acetate solvent. It is explained that the polarity level are playing major role in extracting the secondary metabolites product (Ghasemzadeh, 2011; Senguttuvan *et al*., 2014).

Flavonoids are the content of secondary compounds from plants or a hydroxylated phenolic substance synthesized due to a response to microbial infections. Flavonoids can be used as antimicrobial substances against various types of pathogenic microorganisms. This is due to their ability to interact to dissolve with extracellular proteins and can bacterial cell walls damage (Pandey *et al*., 2010; Mishra *et al*., 2013). Flavonoids are the largest group of natural phenolic compounds and polar compounds because they have some hydroxyl groups (Kumar and Pandey, 2013).

From the results of phytochemical analysis, the best solvent is hexane. Therefore, the extract of *D. salina* with hexane solvent produces the highest content of terpenoids. One of the terpenoids derivatives can be antibacterial found in *D. salina* is β–carotene. It can be seen in FTIR analysis. The mayor pigments present in microalgae to act effectively inhibiting bacteria and microbial growth is chlorophyll and β–carotene (Bhagavathy *et al.*, 2011).

FTIR (Fourier Transform Infrared Spectroscopy) analysis

Based on the FTIR analysis curve (Figure 1), it can be seen that some secondary metabolites contained in *D. salina*. Wave number absorption spectrum is shown in Fig. 1 and the lists of functional groups identified were shown in Table 4.

E-ISSN 2355-9926

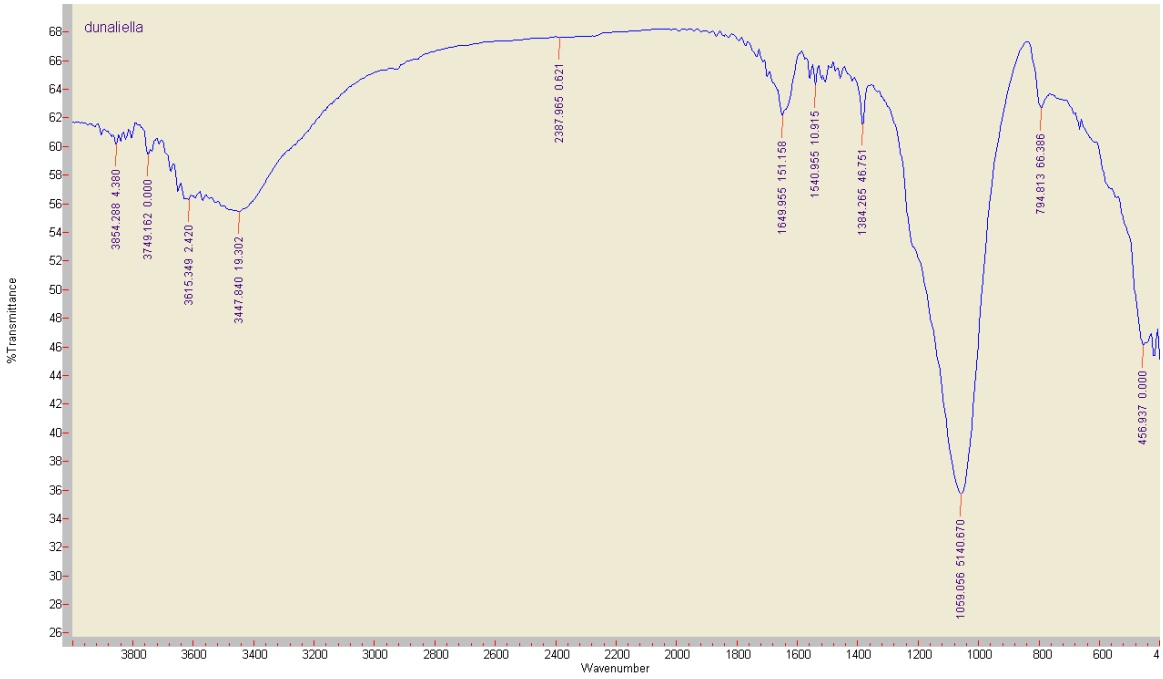

Figure 1. FTIR of Marine Microalgae *D. salina*.

Table 4. The Result FTIR analysis of *D. salina*

IR frequency (cm^{-1})	Bond	Functional groups	*D. salina* frequency (cm^{-1})
3500-3200	O–H stretch, H–bonded	Alcohols, phenols	3447
1670-1600	C=O stretch	Amides	1649
1500-1400	C–C stretch	Aromatics	1540
1400-1300	N=O bend	Nitromethane	1384
1300-1000	C–O stretch	Alcohols, carboxylic acids	1059
850-550	C–H stretch	Alkenes, Aromatics	794

The hexane extract of *D. salina* showed the peaks at 456 cm^{-1}, 794 cm^{-1}, 1059 cm^{-1}, 1384 cm^{-1}, 1540 cm^{-1}, 1649 cm^{-1}, 2387 cm^{-1}, 3447 cm^{-1}, 3615 cm^{-1}, 3749 cm^{-1} and 3854 cm^{-1}. In the area of 3447.84 cm^{-1} indicating the presence of O-H groups, 1649.95 cm^{-1} as C=O group, peak at 1540.95 cm^{-1} indicates the presence of a C-C stretch aromatics group, peak at 1384.26 cm^{-1} shows N=O bend, peak at 1059.05 cm^{-1} shows an C-O functional group of alcohol or carboxylic acids, and the pick emergence of 794.81 cm^{-1} uptake shows the C-H aromatic.

FTIR spectrum of crude extract from *D. salina*, showed similarity with beta carotene in fingerprinting patterns of peaks in 1059 cm^{-1}. It indicates that extracted *D. salina* is a beta carotene or carotenoid derivative (Trivedi *et al.*, 2017; Hosseini *et al.,* 2017). In another peak, analysis of pigment highlighted the stretching of different functional groups with peaks at 1649 cm^{-1}. FTIR peaks in the range of 1600 to 1670 correspond to protein (Suresh, *et al*., 2016).

Hexane extract of *D. salina* showed antimicrobial activity against some bacteria and that this antimicrobial activity might result from fatty acids such as palmitic, α-linolenic, and oleic acids, which are a major component of the extracts (Herrero *et al.*, 2006; Sudalayandi *et al*.,

E-ISSN 2355-9926

2012). In the other research Krishnika *et al*. (2011), identified the antimicrobial activity of different extracts of eight microalgae, and reported that *Dunaliella* sp. showed a high degree of inhibitory effect on *Shigella boydii*, *Salmonella paratyphi* and *Pseudomonas fluorescens*.

Conclusions and suggestions

The results of this study concluded that the *D. salina* extracts contains phenol compounds as antibacterial function. Minimum inhibitory concentration test results obtained 100 $mg.L^{-1}$ which is a minimum concentration that can inhibit the growth of *Edwardsiella tarda*. From FTIR analysis of *D. salina* extract indicate phenols dan terpenoid compounds as a beta caroten. The results of antimicrobial testing of *D. salina* provide valuable information and highlight the potentiality source of antimicrobial agents. Further research on the application of *D. salina* extract to aquaculture organisms infected with *E. tarda* was suggested.

Acknowledgements

The research supported by Indonesia Endowment Fund for Education (LPDP) the Ministry of Finance of the Republic of Indonesia which supports the research through the scholarship and research funding.

References

Anusha, P., and R. S. Bai. (2017). Phytochemical profile and antimicrobial potential of methanolic extracts of bark and leaf of Quassia indica (Gaertn) Nooteb. *The Journal of Phytopharmacology*, *6*(5), 269–276.

Austin, B., and D. A. Austin. (2016). *Bacterial Fish Patogens* (Sixth). United Kingdom: Springer International Publishing Switzerland. https://doi.org/10.1007/978-3-319-32674-0

Bashir, K. M. I., J. H. Lee, M. J. Petermann, A. A. Shah, S. J. Jeong, M. S. Kim, N. G. Park and M. G. Cho. (2018). Estimation of antibacterial properties of chlorophyta, rhodophyta and haptophyta microalgae species. *Microbiology and Biotechnology Letters*, *46*(3), 225–233. https://doi.org/10.4014/mbl.1802.02015

Bbosa, G. S., G. Wong, D. B. Kyegombe and J. O. Okeng. (2014). Effects of intervention measures on irrational antibiotics/antibacterial drug use in developing countries: A systematic review. *Health*, *06*(02), 171-187.https://doi.org/10.4236/health.2014.62027

Bhagavathy, S., P. Sumathi and M. Madhushree. (2011). Antimutagenic assay of carotenoids from green algae *Chlorococcum humicola* using *Salmonella typhimurium* TA98, TA100 and TA102. *Asian Pacific Journal of Tropical Disease*, *1*(4), 308–316. https://doi.org/10.1016/S2222-1808(11)60073-X

Bhakuni, D. S. and D. S. Rawat. (2005). *Bioactive marine natural products*. *Bioactive Marine Natural Products*. https://doi.org/10.1007/1-4020-3484-9

Bhat, V. B. and K. M. Madyastha (2000). C-Phycocyanin: A potent peroxyl radical scavenger in vivo and in vitro. *Biochemical and Biophysical Research Communications*, *275*(1), 20-25. https://doi.org/10.1006/bbrc.2000.3270

Cakmak, Y. S., M. Kaya and M. A. Ozusaglam. (2014). Biochemical composition and bioactivity screening of various extracts

E-ISSN 2355-9926

from *Dunaliella salina*, a green microalga. *EXCLI Journal*, *13*, 679–690.

Candrasari, A., M. A. Romas, M. Hasbi and O. R. Astuti. (2012). Uji daya antimikroba ekstrak etanol daun sirih merah (Piper Crocatum Ruiz & Pav.) terhadap pertumbuhan *Staphylococcus aureus* ATCC 6538, *Eschericia coli* ATCC 11229 dan Candida. *Biomedika*, *4*(1), 9–16.

Cao, L., W. Wang, Y. Yang, C. Yang, Z. Yuan, S. Xiong and J. Diana. (2007). Environmental impact of aquaculture and countermeasures to aquaculture pollution in China. *Environmental Science and Pollution Research*, *14*(7), 452–462. https://doi.org/10.1065/espr2007.05.426

Cardozo, K. H. M., T. Guaratini, M. P. Barros, V. R. Falcão, A. P. Tonon, N. P. Lopes, S. Campos, M. A. Torres, A. O. Souza, P. Colepicolo and E. Pinto. (2007). Metabolites from algae with economical impact. *Comparative Biochemistry and Physiology, 146*, 60–78. https://doi.org/10.1016/j.cbpc.2006.05.007

Davis, W. W. and T. R. Stout. (1971). Disc plate method of microbiological antibiotic assay. *Applied Microbiology*, *22*(4), 659-665.

Devi, T. B., T. J. Abraham and D. Kamilya. (2016). Susceptibility and pathological consequences of catla, *Catla catla* (Hamilton) experimentally infected with *Edwardsiella tarda*. *Archives of Polish Fisheries*, *24*(4), 209–217. https://doi.org/10.1515/aopf-2016-0018

Ely, R., T. Supriya and C. G. Naik. (2004). Antimicrobial activity of marine organisms collected off the coast of South East India. *Journal of Experimental Marine Biology and Ecology*, *309*(1), 121–127. https://doi.org/10.1016/j.jembe.2004.03.010

Evans, W. C. (2002). *Pharmacognosy*. *Fifteenth Edition*. https://doi.org/10.1016/B978-0-7020-2933-2.00017-4

Falaise, C., C. François, M. A. Travers, B. Morga, J. Haure, R. Tremblay, F. Turcotte, P. Pasetto, R. Gastineau, Y. Hardivillier, V. Leignel and J. L. Mouget. (2016). Antimicrobial compounds from eukaryotic microalgae against human pathogens and diseases in aquaculture. *Marine Drugs*, *14*(9), 1–27. https://doi.org/10.3390/md14090159

Firma, A. R. R., U. Sari, C. Chusbul and A. Amri. (2012). Detection of *Edwardsiella tarda* in catfish (*Clarias* sp.) by fluorescent antibody technique (FAT). *Jurnal Akuakultur Indonesia*, *11*(1), 96–102.

Ghasemzadeh, A. and N. Ghasemzadeh. (2011). Flavonoids and phenolic acids: Role and biochemical activity in plants and human. *Journal of Medicinal Plants Research*, *5*(31), 6697–6703. https://doi.org/10.5897/jmpr11.1404

Guedes, A. C., H. M. Amaro and F. X. Malcata. (2011). Microalgae as sources of carotenoids. *Marine Drugs*, *9*(4), 625–644. https://doi.org/10.3390/md9040625

Herrero, M., L. Jaime, P. J. Martín-Álvarez, A. Cifuentes and E. Ibáñez. (2006). Optimization of the extraction of antioxidants from *Dunaliella salina* microalga by pressurized liquids. *Journal of Agricultural and Food Chemistry*, *54*(15), 5597–5603. https://doi.org/10.1021/jf060546q

E-ISSN 2355-9926

Hosseini, S. R. P., O. Tavakoli and M. H. Sarrafzadeh. (2017). Experimental optimization of SC-CO2 extraction of carotenoids from *Dunaliella salina*. *Journal of Supercritical Fluids*, *121*, 89–95. https://doi.org/10.1016/j.supflu.2016.11.006

Jafari, S., M. A. Mobasher, S. Najafipour, Y. Ghasemi, M. Mohkam, M. A. Ebrahimi and N. Mobasher. (2018). Antibacterial potential of *Chlorella vulgaris* and *Dunaliella salina* extracts against *Streptococcus mutans*. *Jundishapur Journal of Natural Pharmaceutical Products*, *13*(2). https://doi.org/10.5812/jjnpp.13226

Jannata, R. H., A. Gunadi and T. Ermawati. (2014). Antibacterial activity of manalagi apple peel (*Malus sylvestris* Mill.) extract on the growth of *Streptococcus mutans*, *2*(1), 23–28.

Jin, R., Y. Hu, B. Sun, X. Zhang and L. Sun. (2012). *Edwardsiella tarda* sialidase: Pathogenicity involvement and vaccine potential. *Fish and Shellfish Immunology*, *33*(3), 514–521. https://doi.org/10.1016/j.fsi.2012.06.002

Kadlec, K., E. V. Duijkeren, J. A. Wagenaar and S. Schwarz. (2011). Molecular basis of rifampicin resistance in methicillin-resistant Staphylococcus pseudintermedius isolates from dogs. *Journal of Antimicrobial Chemotherapy*, *66*(6), 1236–1242. https://doi.org/10.1093/jac/dkr118

Kathleen, M. M., L. Samuel, C. Felecia, E. L. Reagan, A. Kasing, M. Lesley and S. C. Toh. (2016). Antibiotic Resistance of Diverse Bacteria from Aquaculture in Borneo. *International Journal of Microbiology*, *2016*, 1-9. https://doi.org/10.1155/2016/2164761

Keskin, D., D. Oskay and M. Oskay. (2010). Antimicrobial activity of selected plant spices marketed in the West Anatolia. *International Journal of Agriculture and Biology*, *12*(6), 916–920.

Kholil, I., M. M. M. Hossain, M. S. Neowajh, M. S. I. Islam and M. Kabir. (2015). Comparative efficiency of some commercial antibiotics against Pseudomonas infection in fish. *International Journal of Fisheries and Aquatic Studies, 2*(23), 114–117.

Kokou, F., P. Makridis, M. Kentouri and P. Divanach. (2012). Antibacterial activity in microalgae cultures. *Aquaculture Research*, *43*(10), 1520–1527. https://doi.org/10.1111/j.1365-2109.2011.02955.x

Krishnika, A., P. B. Bhanupriya and B. B. Nair. (2011). Antibacterial activity of eight marine microalgae against a few gram negative bacterial pathogens. *Journal of Pharmacy Research*, *4*(9), 3024–3026.

Kumar, S. and A. K. Pandey. (2013). Chemistry and Biological activities of flavonoids: an overview. *The Scientific World Journal*, 1–16. https://doi.org/Volumhttp://dx.doi.org/10.1155/2013/162750

Labh, S. N. and S. R. Shakya. (2014). Application of immunostimulants as an alternative to vaccines for health management in aquaculture. *Nternational Journal of Fisheries and Aquatic Studies*, *2*(1), 153–156.

Lamers, P. P., C. C. W. van de Laak, P. S. Kaasenbrood, J. Lorier, M. Janssen, R. C. H.

E-ISSN 2355-9926

D. Vos, R. J. Bino, R. H. Wijffels. (2010). Carotenoid and fatty acid metabolism in light-stressed *Dunaliella salina*. *Biotechnology and Bioengineering*, *106*(4), 638–648. https://doi.org/10.1002/bit.22725

Li, J., z. Mo, G. Li, P. Xiao and J. Huang. (2015). Generation and evaluation of virulence attenuated mutants of Edwardsiella tarda as vaccine candidates to combat edwardsiellosis in flounder (Paralichthys olivaceus). *Fish and Shellfish Immunology*, *43*(1), 175–180. https://doi.org/10.1016/j.fsi.2014.12.018

Liu, X., J. C. Steele and X. Z. Meng. (2017). Usage, residue, and human health risk of antibiotics in Chinese aquaculture: A review. *Environmental Pollution*, *223*, 161–169. https://doi.org/10.1016/j.envpol.2017.01.003

Mehana, E., A. Rahmani and S. Aly. (2014). Immunostimulants and Fish Culture: An Overview. *Annual Research & Review in Biology*, *5*(6), 477–489. https://doi.org/10.9734/arrb/2015/9558

Mendes, R. L., B. P. Nobre, M. T. Cardoso, A. P. Pereira and A. F. Palavra. (2003). Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae. *Inorganica Chimica Acta*, *356*, 328–334. https://doi.org/10.1016/S0020-1693(03)00363-3

Mishra, A., A. K. Sharma, S. Kumar, A. K. Saxena and A. K. Pandey. (2013). Bauhinia variegata leaf extracts exhibit considerable antibacterial, antioxidant, and anticancer activities. *BioMed Research International*, *2013*. https://doi.org/10.1155/2013/915436

Narwiyani, S. and K. Kurniasih. (2011). Pathogenicity comparison of *Edwardsiella tarda* infection in goldfish (*Charassius auratus*) and celebes rainbow (*Telmatherina celebensis*). *Jurnal Riset Akuakultur*, *6*(2), 291–301. https://doi.org/10.15578/jra.6.2.2011.291-301

Ogbonne, F. C., E. R. Ukazu and F. C. Egbe. (2018). Antibiotics resistance pattern and plasmid profiling of Edwardsiella tarda isolated from Heterobranchus longifilis. *Journal of Biosciences and Medicines*, *06*(04), 95–105. https://doi.org/10.4236/jbm.2018.64008

Pandey, A. K., A. K. Mishra, A. Mishra, S. Kumar and A. Chandra. (2010). Therapeutic potential of *c. zeylanicum* extracts : an antifungal and antioxidant perspective. *International Journal of Biological and Medical Research*, *1*(4), 228–233.

Park, S. B., T. Aoki and T. S. Jung. (2012). Pathogenesis of and strategies for preventing Edwardsiella tarda infection in fish. *Veterinary Research*, *43*(1), 1–11. https://doi.org/10.1186/1297-9716-43-67

Pasquet, V., J. R. Chérouvrier, F. Farhat, V. Thiéry, J. M. Piot, J. B. Bérard, R. Kaas, B. Serive, T. Patrice, J. P. Cadoret and L. Picot. (2011). Study on the microalgal pigments extraction process: Performance of microwave assisted extraction. *Process Biochemistry*, *46*(1), 59–67. https://doi.org/10.1016/j.procbio.2010.07.009

Prasad, N. D., B. G. Rao, B. S. Rao, T. M. Rao and D. V. S. Praneeth. (2012). Quantification of

E-ISSN 2355-9926

phytochemical constituents and in-vitro antioxidant activity of Mesua ferrea leaves. *Asian Pacific Journal of Tropical Biomedicine*, *2*, S539–S542. https://doi.org/10.1016/S2221-1691(12)60269-X

Qiao, M., G. G. Ying, A. C. Singer and Y. G. Zhu. (2018). Review of antibiotic resistance in China and its environment. *Environment International*, *110*, 160–172. https://doi.org/10.1016/j.envint.2017.10.016

Senguttuvan, J., S. Paulsamy, and K. Karthika. (2014). Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, Hypochaeris radicata L. for in vitro antioxidant activities. *Asian Pacific Journal of Tropical Biomedicine*, *4*(1), 359–367. https://doi.org/10.12980/apjtb.4.2014c1030

Shin, D. M., S. Hossain, SHMP Wimalasena and G. J. Heo. (2017). Antimicrobial resistance and virulence factors of Edwardsiella tarda isolated from pet turtles. *Pakistan Veterinary Journal*, *37*(1), 85–89. https://doi.org/10.1097/QCO.0b013e3283638104

Sudalayandi, K., A. Kumar, R. Sessler, R. T. Sayre, V. Falcoa, U. Ihemere, J. Ndunguru and N. Narayanan. (2012). Determination of fatty acids and proteins from the fresh water alga Chlamydomonas reinhardtii CC 2137 and its antagonism against aquatic bacteria. *Pakistan Journal of Botany*, *44*(6), 2139–2144.

Surendhiran, D., M. Vijay, A. R. Sirajunnisa, T. Subramaniyan, A. S. Shellomith and K. Tamilselvam. 2014. A green synthesis of antimicrobial compounds from marine microalgae Nannochloropsis oculata. *Journal of Coastal Life Medicine*, *2*(11), 859–863. https://doi.org/10.12980/jclm.2.2014apjtb-2014-0138

Suresh, S., S. Karthikeyan and K. Jayamoorthy. (2016). FTIR and multivariate analysis to study the effect of bulk and nano copper oxide on peanut plant leaves. *Journal of Science: Advanced Materials and Devices*, *1*(3), 343–350. https://doi.org/10.1016/j.jsamd.2016.08.004

Syafitrianto, I., A. Aqmal and M. N. H. Lande. (2016). Variasi Aeromonas pada ikan sidat (*Anguilla* sp.) yang dilalulintaskan melalui bandar udara palu. *Biogenesis: Jurnal Ilmiah Biologi*, *4*(1), 10–15. https://doi.org/10.24252/bio.v4i1.1114

Syed, S., A. Arasu and I. Ponnuswamy. (2015). The uses of Chlorella vulgaris as antimicrobial agent and as a diet: The presence of bio-active compounds which caters the vitamins, minerals in general. *International Journal of Bio-Science and Bio-Technology*, *7*(1), 185–190. https://doi.org/10.14257/ijbsbt.2015.7.1.19

Trivedi, N., S. Tandon and A. Dubey. (2017). Fourier transform infrared spectroscopy (FTIR) profiling of red pigment produced by Bacillus subtilis PD5. *African Journal of Biotechnology*, *16*(27), 1507–1512. https://doi.org/10.5897/ajb2017.15959

E-ISSN 2355-9926