

Eucalyptus camaldulensis Extract as a Preventive to the Vibriosis in Western White Shrimp (*Litopenaeus vannamei*) in Bushehr Province

Ghasemian M*

Microbiology Department, Faculty of Medicine, AJA University of Medical Sciences, Tehran, Iran

Abstract

Objective: In order to prevent the unusual use of antibiotics and promote green aquaculture, researchers have used various herbs as medicines proposed in the aquaculture industry. One of medicinal plants is *Eucalyptus camaldulensis* which has been identified in different studies for the antimicrobial activity of the extract in controlling and eliminating bacterial and fungal strains which was evaluated in this study.

Methods: In this research, a variety of Soxhlet and rotary extracts of ethanol, methanol, acetone and water soluble compounds and its antibacterial effect was investigated by agar diffusion method in a well. For this purpose, *Vibrio Harveyi* and *Vibrio azureus* (KJ018724.1) were isolated and identified by the sequencing of 16SrDNA.

Results: The results showed that 2.5 µg/ml extracts of ethanol, methanol, acetone and 10 µg/ml of aqueous extract of the herb had minimum inhibitory concentration (MIC) and also 10 µg/ml of ethanol, methanol, acetone and 40 µg/ml of aqueous extract conferred minimum bacterial concentration (MBC) against *V. harveyi* in addition, 5 µg/ml of each methanolic and acetone, 2.5 µg/ml of ethanolic and 10 µg/ml of aqueous extracts had MIC on *V. azureus* and 5 µg/ml of ethanolic and 10 µg/ml of methanolic and acetone and 20 µg/ml of aqueous extracts showed MBC against *V. azureus*. Moreover, by examining the kinetics of *V. harveyi* and *V. azureus*, the *Eucalyptus camaldulensis* extract had growth inhibitory effect on the both species.

Conclusion: The effects of various environmental factors such as temperature, salinity and time on the antimicrobial activity, the extract exhibited a good performance and can be used as an environmentally proper and compatible antimicrobial agent in ecological conditions and shrimp breeding areas, especially in southern Iran.

Keywords: Vibriosis; *Vibrio azureus*; *Vibrio harveyi*; *Eucalyptus camaldulensis*

Introduction

Health management Pathogenic factors in the shrimp breeding industry are based on three axes: diagnosis, prevention and treatment. Due to the increased frequency of microorganisms in artificial growth environments, diseases have also been increasing and causing irreparable damage to the industry [1]. Among those important types of pathogens there are bacterial and viral agents. One of the most important types of these bacteria in the marine ecosystems is Vibrionaceae family [2]. These bacteria are a natural flora in the marine ecosystem, which, in the second place under certain conditions, such as stress, vitamin C deficiency, high density, algae, viral diseases, etc., cause the disease of vibriosis in aquatic organisms. On the other hand, the untapped use of chemical drugs and, consequently, the increasing resistance of pathogens, necessitate the need for new compounds and drugs more urgently. An overwhelming use of antibiotics as a growth promoter has led to the development of resistant strains [3]. This problem has been observed remarkably in the shrimp breeding industry, since large amounts of antimicrobial compounds have been used in dairy farming systems that have led to the spread of antibiotic-resistant bacterial strains [4]. The risk of developing antibiotic-resistant strains is important not only for aquaculture but also for human health, and there are many reports that antibiotic resistance genes are transferable among bacteria. Today, the tendency towards no overuse of antimicrobials, the use of alternative curative approaches is also increasing. Alternatives to these treatments are the use of new ways, such as probiotic bacteria or natural antibacterial compounds [5]. For the production of new drugs, various sources, especially plants, are of interest to researchers. Essences in aromatic plants are a valuable category of natural compounds with various medicinal properties,

including antimicrobial properties. Examples of these *Eucalyptus* plants [6] Clove, and others [7].

Therefore, in this study was designed to evaluate the antimicrobial properties of *Eucalyptus camaldulensis* extract on the prevention of bacterial contamination including Vibrionaceae family. Due to the ecological conditions of shrimp breeding areas especially in the south of the country that have specific climate conditions, the shrimp breeding in Iran is different from other countries. The biotic activity of the extract of this plant in different conditions of temperature, salinity and different time spans was investigated.

Materials and Methods

In this study, from March to June 2013 (growth period), for the determination of the predominant bacterium in shrimp propagation, 12 main shrimp tanks were sampled and, in total, given three replicates per Sampling times, 108 sources were sampled and evaluated for abundance and bacterial diversity.

The polyethylene disposable containers were made sterile by gamma rays, sterilized both internally and externally. Regarding the condition of storage tanks, the sampling was carried out at a depth of

*Corresponding author: Ghasemian M, Microbiology Department, Faculty of Medicine, AJA University of Medical Sciences, Tehran, Iran; Tel: +98 21 86094475; E-mail: majidghasemian86@gmail.com

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40-50 cm. After registration of the identification code, and sampling location data and history, transferred away from sunlight in the vicinity of dry ice bags and were kept in the refrigerator of a Laboratory of Microbiology of the Shrimp Research Institute of the country.

Each time, sampling of physicochemical factors of shrimp propagation water, including temperature, salinity and dissolved oxygen, was measured and recorded by portable multi-parameters (HACH model 40d).

Total count of Vibrionaceae family

The total count of the Vibrionaceae family in water was determined according to the total counting of aerobic and anaerobic heterotrophic bacteria, but the thiosulfate-citrate-bile salt-sucrose agar (TCBS) culture medium was used.

Each time the predominant colonies were sampled and cultured, and purified by streak in TSA culture medium. For genus level identification, one of the most common colonies was identified, and for this purpose routine differential diagnosis of bacteria was performed based on biochemical characteristics. These tests included Gram staining, oxidase test, catalase, sugar consumption, arginine dehydrogenase, motility, citrate digestion, growth in various salt concentrations, growth in Macconkey agar, growth in OF medium and O/129 disc sensitivity.

Molecular identification

In order to extract the genomic DNA, the genomic DNA extraction kit for Gram-negative bacteria was purchased from the National Iranian Biosphere Reserve (IBRC). Using this kit, the bacterial cell walls and cell membranes were first decomposed using leaching solutions, and then, by providing suitable conditions for the environment, the DNA was attached to the silica column, two steps of column washing using buffers, washing all impurities from the DNA, and the column was washed and finally, using a soft saline buffer solution, the DNA was isolated from the column and dissolved in the salt buffer, and obtained with a small centrifuge of very pure DNA with a desired amount. In this method, unlike traditional methods, toxic substances such as phenol and chloroform were discarded.

PCR amplification of bacterial 16srDNA gene

According to Table 1, the reaction solutions were prepared. All the solutions in the table were added on the ice, and finally template DNA was added and followed by a vortex of the mixture, and the reaction tubes were placed inside the Thermal Cycler. The sequences of primers included: forward (5-GAGTTTGATCCTGGCTCAG-3) and reverse (ACGGGCGGTG+GTRC). Preparing a herbal compounds.

The new *Eucalyptus camaldulensis* leaves were obtained from the Applied Scientific Training Center of Agricultural Jihad in Bushehr province. After checking the leaves in terms of quality and appearance,

Material	Amount
10X Taq Buffer	0.5 µl
dNTP Mix, 2 mM each	1.0 µl
27F	0.5 µl
1392R	0.5 µl
25 Mm MgCl ₂	1.5µl
Template DNA	50-100 ng
Taq DAN Polymerase	0.5 µl
Water, nuclease-free	up to 50 µl
Total Volume	50 µl

Table 1: PCR reaction tube master mix in this study.

they were transferred into nail bags and clean plastic containers and transferred to the Laboratory of Microbiology of the Shrimp Research Institute of the country. At first, the leaves were washed with fresh water and cleaned on nylon cleaners to dry completely. In the next step, the dried leaves were crushed by the mill and powdered and put in dark containers for extraction and stored in dark, dry and cool conditions.

The condensed extract was obtained using a rotary machine at 40°C for 45 minutes using each of ethanol, methanol, acetone and aqueous extract for 2 hours and then stored in a glass bottle and stored in the refrigerator until use. For aqueous extracts from ratio 1 to 5 and for alcoholic extracts, ratios of 1 to 10 were used. The extracts were concentrated to reach the formula of Moses (product=% of the raw material).

Bacteria isolates

In this study, *V. harveyi* IS 01, PTTC 1755, with GU 974342, 1 registration number in the World Bank,) was previously isolated and purified by the shrimp institute, and *V. azureus* were used.

Antibacterial effects of *Eucalyptus camaldulensis* extracts

To evaluate the antimicrobial effects of *Eucalyptus camaldulensis* extracts on *V. harveyi* and *V. azureus* bacteria, concentrations of 40, 20, 10, 5, 2.5, 1.25 and 0.625 mg/250 ml of aqueous, ethanolic, methanolic and acetone *Eucalyptus camaldulensis* extracts were prepared by two methods, soxhlet and rotary, and tested by agar diffusion method in a well. In this method, Muller Hinton Agar supplemented with 1% Salt was used and overnight cultures of *V. harveyi* and *V. azureus* at a concentration of 0.5 McFarland were inoculated and was lawn using the tip of the sterile pipette Pasteur. Then, 50 µl of the extract was poured into wells and stored at 15°C for 2 hours until all the extracts of the agar were released and finally the plates were incubated at 30°C for 24 hours. The ability of the antimicrobial activity of the extracts was measured and recorded based on the diameter of the inhibition zone of the bacteria (in millimeters). To determine the effect of extract and eliminate the effects of solvent itself, solvents were used as controls.

Minimum inhibitory concentration and minimum bactericidal concentration of *Eucalyptus camaldulensis*: At this stage, the MIC of *Eucalyptus camaldulensis* extract was obtained for the bacteria by serial dilution method, as in a study by Moses and Fors. For this purpose, TSB containing 2.5% salts and 8 tubes, 2 of which for positive control (culture media and bacteria) and negative control (containing *Eucalyptus camaldulensis* extract) were selected. Next, 1 ml of aqueous extract of *Eucalyptus camaldulensis* at a concentration of 0.08 g into a tube containing TSB medium and dilution series at concentrations of 10, 5, 2.5, 1.25, 0.625 and 0.3125 mg/250 ml and bacterial suspension equal to the 0.5 McFarland.

Effect of eucalyptus extracts at different temperatures: For this purpose, 1 ml of *Eucalyptus camaldulensis* extract with 250 mg/ml concentration was poured into sterile microtubes and three replicates were used for each extract and they were covered with sterile liquid paraffin to prevent evaporation. After preparing the treatments, they were placed in a thermoblock and the antibacterial effect of the extract was studied at 35, 40, 45, 55, 65, 75, 85 and 100°C temperatures at 10 and 20 minutes and by the agar diffusion method. In addition, the antimicrobial effect of the extract on bacterial isolates was evaluated at after keeping of extract at 121°C in autoclave conditions.

Antibacterial effect of eucalyptus extract at different times: In order to determine the shelf-life of the selected eucalyptus extracts at different times and different storage conditions (temperature 25°C,

temperature 4°C and temperature-80°C), it was monitored on a monthly basis and the antibacterial properties was assessed with the agar diffusion method.

Antibacterial activity of eucalyptus extract in different saline conditions: For selected Eucalyptus extract, 6 sterile Falcons were considered with three replications. First, by mixing sterile water and sterile distilled water, solutions with different salinity concentrations (44, 50, 60, 78 ppt) and a volume of 5 ml was prepared and next 1:1 of these tubes the eucalyptus extracts were added and the final salinity of the solutions was recorded by the refractometer. After 30 minutes of treatment, the effect of antimicrobial extraction on the bacteria was evaluated and recorded according to the agar diffusion method.

Antibiotic resistance profile of predominant bacterial pathogens: In order to determine the antibiotic resistance profile of the dominant and pathogenic bacteria, antibiotics including ampicillin (30 µg), chloramphenicol (30 µg) Co-trimoxazole (30 µg) and tetracycline (30 µg) were used according to the CLSI guidelines and the Kirby-Bauer method, on MHA agar culture media, and then the bacterial susceptibility table was reported as susceptible, intermediate and resistant to antibiotics.

Data analysis

the data were analyzed using EXCEL 2010 and SPSS 18 software using analytical tests one-way ANOVA and Tukey's and T-test with 95% confidence level ($p < 0.05$ as significant).

Results

Molecular identification of bacteria

Based on the results of 16srDNA molecular analysis of strain isolated using Blast and EZ taxon software, this bacterium belonged to gamma proteobacteria, *Vibrionaceae* family and vibrio genus was 100% similar to *V. azureus*, so the results of the software and the neighbor joining method after the phylogeny tree was mapped with the strain IS012 and the KJ018724.1 number at the World Gen Bank (Figure 1).

Antibacterial effects of different extract of eucalyptus camaldulensis on V. harveyi and V. azureus: The results of this study indicated that the amount of bioavailability of the aqueous extract of

Eucalyptus camaldulensis leaves on *V. azureus* was significantly higher than that of *V. harveyi* ($P < 0.05$).

Furthermore, despite the higher bioavailability of the methanolic extract of *Eucalyptus camaldulensis* leaves on *V. harveyi*, no significant difference was observed compared to *V. azureus* ($P > 0.05$).

The results showed that the bioavailability of the ethanolic extract of *Eucalyptus camaldulensis* leaves on *V. harveyi* was significantly higher than the bioavailability against values of *V. azureus* ($P < 0.05$).

The results of acetone extract showed that despite the higher bioactive values of this extract against *V. harveyi* strain than *V. azureus*, there was no significant difference ($P > 0.05$).

The results showed that the highest biological activity observed against *V. harveyi* was related to acetone extract with $7.08 \times 10^5 \pm 2.01 \times 10^4$ bioactive activity/ml, and the lowest observed was related to aqueous extract with 1.43×10^5 bioactive activity/ml. in addition, there was a significant higher activity for acetone extract, but no significant result was observed compared to ethanolic extract.

MICs of various extracts of Eucalyptus camaldulensis leaf on V. harveyi and V. azureus: According to the results, it was observed that the MICs ethanolic, methanolic, acetone and aqueous extracts of *Eucalyptus camaldulensis* against *V. harveyi* by rotary method was 1.95 mg/ml, while by Soxhlet method, the MICs of ethanol, methanol and acetone extracts was calculated and the highest amount was related to aqueous extract at 7.81 mg/ml.

Meanwhile, the MICs against *V. azureus* growth in each soxhlet and rotary was for ethanolic and methanolic extracts respectively and the highest amount in both extracts was obtained from the extract of *Eucalyptus camaldulensis* leaf with the rate of 8.18 mg/ml.

MIC against *V. harveyi* in both methods was related to ethanolic, methanolic and acetone extracts of *Eucalyptus camaldulensis* leaves, while the aqueous extract had the highest concentration in soxhlet and rotary methods being 31.25 and 15.63 mg/ml, respectively.

Discussion

Concerning the *V. azureus* bacteria, the results showed that the diameter of the inhibition zone of bacteria in exposure to methanolic, ethanolic and acetone extracts of *Eucalyptus camaldulensis* leaves was significantly less than the diameter of the non-growth zone when exposed to chloramphenicol, co-trimoxazole and tetracycline antibiotics ($P < 0.05$). However, the diameter of the non-growth zone of *V. azureus* exposed to the aqueous extracts of *Eucalyptus camaldulensis* leaves was significantly less than other extracts ($P < 0.05$). Given the development of the shrimp industry over the past twenty years, the risk of the spread of some diseases has always threatened the industry. Therefore, the accurate identification and control of the mentioned factors has always been one of the main concerns of shrimp breeding centers. Therefore, according to the results of chemical and molecular experiments, two bacterial strains of *V. harveyi* and *V. azureus* were identified and isolated according to the World Bank No. IS01PTTC1755 and KJ018724.1, respectively. However, today, various methods, including the use of antiseptic chemicals and broad-spectrum antibiotics such as oxytetracycline, are used to control and treat the abovementioned factors, therefore, due to the occurrence of drug resistance and the persistence of these substances in Shrimp tissues have been banned from using this material [8,9]. Today, the use of environmentally friendly materials, such as extracts, essential oils and herbal extracts, is proposed as an effective and safe therapeutic approach [10,11]. On the other hand, these compounds have the

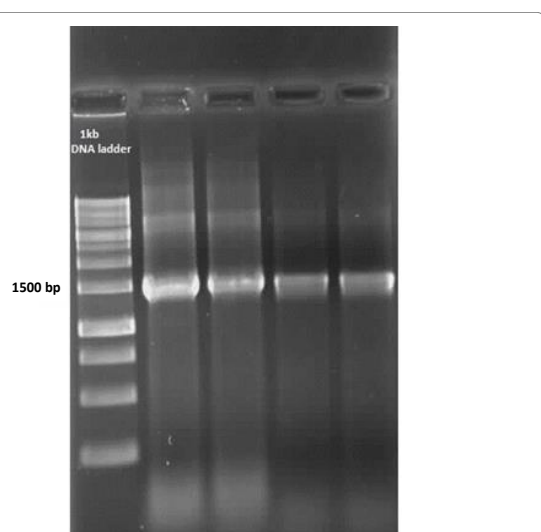


Figure 1: PCR products of 16srDNA with 1500 bp size.

potential to prevent the growth of pathogenic agents by stimulating the immune system, in addition to their antibacterial, fungal, viral and antioxidant properties. Among these materials, the essential oil and extracts extracted from the leaves of the *Eucalyptus* medicinal herb of the *Kamelodolensis* species are mentioned. It is said to belong to the Miratase family and is mostly found in tropical regions of the planet, usually a rich source of polyphenols, terpenes, cineoles, and game compounds. Therefore, in this study, the results of antimicrobial effects of different extracts of *Eucalyptus camaldulensis* leaves on the growth of two bacterial strains of *V. harveyi* and *V. azureus* showed a significant difference between the antibacterial effects of different extracts, such as The extract of acetone extracted from *Eucalyptus camaldulensis* leaf had the highest antibacterial effect compared to aqueous, ethanolic and methanolic extracts.

However, the antibacterial effects of aqueous, ethanolic and methanolic extracts differed from the *Vibrio* strain, so, according to the results, it was observed that the aqueous extract extracted from the leaves of *Eucalyptus Kamelodolensis* leaves compared to methanol and ethanol extracts significantly. The most antibacterial and growth inhibitory effects were on *V. azureus* bacteria, but antibacterial effects of ethanolic and methanolic extracts of *V. harveyi* were higher than the aqueous extract, but no significant differences were observed. Therefore, the main factor of this difference, on the one hand, can be due to the difference in the type and composition of the extracted from the leaves of *Eucalyptus camaldulensis* in the Acetone method in comparison to the water, ethanol and methanol method. Therefore, based on the studies, it was stated that according to the method of extraction and growing area of the *Eucalyptus kamalodulensis* plant, the extracted compounds and extracts can vary. Therefore, Cian stated that the most important extracts from *Eucalyptus camaldulensis* leaves are ethanolic and blue methods, including polyphenols, terpenoids, eucalyptol or cineol, and the composition of the game is 70-80 milligrams. Meanwhile, saponin, saponin glycoside, steroid, cardiac glycoside, tannin, volatile oils, phenols, oclibus, grundinole, macrocarpal, ovalimine, robustadial, 1-8 inulin and eucalypton compounds extracted from *Eucalyptus camaldulensis* leaves by method Methanolic. It is worth noting that all of the above compounds have antiviral, bacterial and fungal effects. However, Moghimi stated that among the compounds mentioned above, the most extracted from the leaves of *Eucalyptus camellodolensis* 1 and 8 cineol, which has high antibacterial properties. Therefore, due to differences in the type and amount of the extracted compounds from each leaf of *Eucalyptus camaldulensis*, which was used in the study in each of the methods for extracting this difference, may have been created.

On the other hand, in addition to the above, the differences in sex and bacterial species can also affect the amount of antibacterial effects of different extracts of *Eucalyptus camaldulensis*. It is stated that due to the difference in the cell wall structure of gram positive and gram negative bacteria, the antibacterial effects of the leaf extract of *Eucalyptus camaldulensis* leaves are different. Alizadeh noted that the antibacterial effects of alcoholic and aqueous extracts of *Eucalyptus camaldulensis* leaves on Gram-positive bacteria, like *Staphylococcus aureus*, are higher than that of the Gram-negative bacterium *Escherichia coli*. Therefore, considering that the two bacterial species used in this study were gram negative bacteria, the results showed that the minimum inhibitory concentration of *Vibrio harveyi* bacteria in the concentration of 1.95 mg/ml, for ethanolic, estine and methanol extracts. The leaves of *Eucalyptus camaldulensis* were obtained, while the minimum inhibitory concentration of growth for *Vibrio azureus* bacterium was extracted for ethanolic and methanol extracts by Soxhlet

and Rotary method at a concentration of 1.95 mg/ml and a minimum concentration of fecundity for *Vibrio harveyi* and *Vibrio azureus* is 81.8 mg/ml of extract. Of methanol and Acetone extracted by the Laboratory. However, at least the calculated fecundity of *Vibrio Harveyi* bacteria for ethanolic, methanol and estrogen extracts was obtained by rotary method of 3.91 mg/ml and in the case of *Vibrio azureus* 1.95 mg/ml for methanol extract. Therefore, due to the difference in the structure of the germ cell wall of the gram-positive and gram-negative bacteria, the amount of mucopeptide compounds in the cell wall of the bacterium is more gram-positive, whereas in the gram-negative bacterium, only a thin layer of mucopeptide is located, and a large part of the cell wall structure. They consist of lipoprotein and lipopolysaccharide.

It is worth noting that, despite the fact that the extract of Acetone compared to other methods used in this study showed an antibacterial effect, but due to the safety and ease of extraction of the extract from *Eucalyptus camaldulensis* leaves by aqueous method, aqueous extract of leaf *Eucalyptus camaldulensis* was used to determine the bioavailability of two bacterial strains of *Vibrio harveyi* and *azureus* in different conditions. The results showed that the highest bioavailability of the aqueous extract of *Eucalyptus camaldulensis* leaves in both species of vibrio at low temperatures, so that with increasing temperature, this activity gradually decreases, but it can be noted that the increase in temperature may, in addition to breaking some of the chemical bonds and destroying some of the extracted compounds, reduce the amount of bioavailability and antibacterial effects of the aqueous extract. Therefore, it is recommended that if this extract is supplemented with food, it should be added to the food after cooking shrimp. Also, according to the results, the best bioavailability of the aqueous extract of *Eucalyptus camaldulensis* plant at +4°C temperature would be maximal for two months. Therefore, if this extract is coated with food, the maximum time it can be stored at +4°C is two months, but after this time and beyond this temperature range of bioavailability the aqueous extract will be reduced.

Due to the saltiness of the two bacterial species, *V. harveyi* and *V. azureus* used in this study, these two species are capable of causing pathogens in the pasture environments. On the other hand, considering that *Eucalyptus camaldulensis* B has high salinity tolerance, it can easily grow in saline and dry areas. Therefore, in this study, the degree of salinity of the extracted water extracted from the *Eucalyptus kamalodolensis* plant is 22 parts per thousand. Therefore, considering that the most pathogenic activity of *Vibrio* bacteria occurs in salinity higher than 35 ppm, the results of determination of bioavailability at different salinity levels indicate that with increasing salinity levels, the bioavailability of the aqueous extract of this plant will increase in such a way that the maximum bioavailability observed in salinity levels is higher than 60 ppm. Also, considering that both bacterial species used in the study at high salinity levels could be pathogenic, there was no significant difference in bioavailability of the aqueous extract in both species of *Vibrio* bacteria in different salinity levels. Therefore, due to the fact that salinity of water in shrimp farms in Bushehr province is in the range of 55-45 parts per thousand, but the use of this extract in the above-mentioned breeding grounds can be effective.

On the other hand, due to the presence of compounds like cinnamyl 1 and 8 in extracts and essential oils of medicinal plants, especially the leaves of *Eucalyptus camaldulensis*, due to their hydrophobic properties, the mechanism of antibacterial effect of these compounds is that by the release of cell wall lipid molecules. The bacteria increase the permeability of the cell membrane and rupture the cell wall structure, leaving many of the ions and compounds in the bacterial cell out of the cell and ultimately leading to cell death.

Also, despite the high sensitivity of *Vibrio harveyi* and *Vibrio azureus* to the antibiotics of chloramphenicol and cotrimoxazole, the results of antibiogram profile showed that the susceptibility of these two species to the estrogen and methanolic extracts is semi-sensitive. Therefore, considering the development of drug resistance following the use of antibiotics and the preservation of the drug in the tissue of living organisms, it can be noted that due to the antibacterial properties of the extracts extracted from the leaves of *Eucalyptus camaldulensis* and the susceptibility of the bacteria *Vibrio harveyi* and *azureus* Extract of *Eucalyptus camaldulensis* leaves to prevent bacterial growth.

Conclusion

It can be concluded that acetone, ethanol, methanol and aqueous extracts from *Eucalyptus camaldulensis* leaves have efficient antibacterial effects on *V. harveyi* and *V. azureus* species, so that the extract of acetone had the highest antibacterial effect compared to other extracts. However, due to its safe and ease of use, it was used to extract the aqueous extract. The highest bioavailability of aqueous extract was obtained at +4°C for a maximum of two months. On the other hand, it was observed that increasing the salinity level of aqueous extract increased the bioavailability of the extract. It was also observed that the antibacterial effects of aqueous extract on *V. harveyi* bacteria were higher than that of *V. azureus*. Since *V. azureus* is a species that has the highest frequency in water, it can be noted that the use of the Eucalyptus aqueous extract is an appropriate replacement for iodine and other chemicals used for treatment of water and materials before propagation of the breeders to the tank and can be highly effective in preventing the growth of *Vibrio* species.

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References

1. Suárez-Abelenda M, Ferreira T, Camps-Arbestain M, Rivera-Monroy V, Macías F, et al. (2014) The effect of nutrient-rich effluents from shrimp farming on mangrove soil carbon storage and geochemistry under semi-arid climate conditions in northern Brazil. *Geoderma* 213: 551-559.
2. Wahjuningrum D, Tarman K, Effendi I (2016) Feeding duration of dietary *Nodulisporium* sp. KT29 to prevent the infection of *Vibrio harveyi* on Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture, Aquarium, Conservation & Legislation. International Journal of the Bioflux Society (AAFL Bioflux)* 9: 1265-1277.
3. Pang B, Du P, Zhou Z, Diao B, Cui Z, et al. (2016) The transmission and antibiotic resistance variation in a multiple drug resistance clade of *Vibrio cholerae* circulating in multiple countries in Asia. *PLoS One* 11: e0149742.
4. Jacoby GA (2017) Plasmid-mediated quinolone resistance. *Antimicrobial Drug Resistance. Springer*: 265-268.
5. Czaplewski L, Bax R, Clokie M, Dawson M, Fairhead H, et al. (2016) Alternatives to antibiotics—a pipeline portfolio review. *Lancet Infect Dis* 16: 239-251.
6. Hyldgaard M, Mygind T, Meyer RL (2012) Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. *Front Microbiol* 3.
7. Fu Y, Zu Y, Chen L, Shi X, Wang Z, et al. (2007) Antimicrobial activity of clove and rosemary essential oils alone and in combination. *Phytotherapy Research* 21: 989-994.
8. Noriega-Orozco L, Acedo-Félix E, Higuera-Ciajara I, Jiménez-Flores R, Cano R (2007) Pathogenic and non pathogenic *Vibrio* species in aquaculture shrimp ponds. *Rev Latinoam Microbiol* 49: 60-67.
9. Mohamadi M, Zamini AA, Vahabzadeh H (2013) Evaluation of antibacterial properties of eucalyptus spp and plelargonium roseum extracts in common carp, cyprinus carpio and their effect on blood indices. *Middle-East Journal of Scientific Research* 15: 723-731.
10. Williams C (2011) Medicinal plants in Australia volume 2: gums, resins, tannin and essential oils, rosenberg publishing.
11. Zandi K, Ramedani E, Mohammadi K, Tajbakhsh S, Deilami I, et al. (2010) Evaluation of antiviral activities of curcumin derivatives against HSV-1 in Vero cell line. *Natural product communications* 5: 1935-1938.