**World Journal of Pharmaceutical Sciences**
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers 
Available online at: http://www.wjpsonline.org/
**Original Article**

# Antibacterial activity of *Eucalyptus oblique* aqueous leaf extract on selective *Vibrio* species of *Penaeus monodon* culture hatchery

A. Sivaraj [1]*, J. Devagnanaroopan[1], B. Palani[2], B. Senthilkumar[1]

[1]Department of Zoology, Thiruvalluvar University, Serkkadu, Vellore – 632 115. Tamilnadu, India.
[2]Department of Zoology, Government Thirumagal Mills College, Gandhi Nagar, Guidiyattam – 632 602. Tamilnadu. India.

***Received: 06-11-2016 / Revised: 23-12-2016 / Accepted: 26-12-2016 / Published: 01-01-2017***

## ABSTRACT

The anti-bacterial activity of aqueous leaf extract of *Eucalyptus oblique* against selective *Vibrio* spp was evaluated. *Vibrios* are marine bacterium, responsible for disease outbreak affecting commercial production of shrimp species especially *Penaeus monodon*. *Vibrios* were cultivated on Thiosulfate-citrate-bile salts-sucrose (TCBS) agar and seven colonies of *vibrio* spp. were isolated based on their colony morphology. The aqueous plant extract was prepared from *Eucalyptus oblique* and its antibacterial activity evaluated against selected *Vibrio* spp. The antibacterial activity was tested by using the disk diffusion method, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) methods.

**Keywords:** TCBS, MIC, MBC, *Eucalyptus oblique*, *Penaeus monodon*

## INTRODUCTION

Aquaculture involves cultivating freshwater and saltwater populations under controlled conditions and can be contrasted with commercial fishing, which is the harvesting of wild fish. Broadly speaking, finfish and shellfish fisheries can be conceptualized as akin to hunting and gathering while aquaculture is akin to agriculture [1].

The importance of the fisheries sector in India is demonstrated by the fact that it employs more than five million people [2], contributes to food and nutritional security and employment, supports livelihoods, and raises the socioeconomic status of poor fishing communities. During the past half-century, Indian fish production registered excellent growth, from a meager 0.75 m t in 1950 to 6.3 m t in 2002 [2,3]. The sector is one of the major contributors to foreign exports. During the past two decades, the inland fisheries in India, which include both capture and culture fisheries, have registered tremendous growth and change. Almost 400 species are reared in the aquatic environment with the aim of harvesting animal or plant protein.

Commercial shrimp farming began in the 1970s, and production grew steeply thereafter. Global production reached more than 1.6 million tonnes in 2003, worth about 9 billion U.S. dollars. About 75% of farmed shrimp is produced in Asia. Infectious diseases pose one of the most significant threats to successful shrimp culture. The Industrial monocultures are very susceptible to disease, which has decimated shrimp populations across entire regions. Cultured shrimps suffer from various diseases due to infectious and non-infectious causes. Infectious diseases are caused by viruses, bacteria, fungi and certa*in* parasites.

*Vibriosis*, is the bacterial disease caused by *Vibrio* spp. It is one of the most prevalent diseases in aquaculture-reared organisms and widely responsible for mortality in shrimp culture worldwide [4,5]. Treatment of bacterial diseases with different herbs has been safely used in organic agriculture, veterinary and human medicine [6], and treatments with medicinal plants having antibacterial activity are a potentially beneficial alternative in aquaculture [7,8].

Therefore, due to increasing the resistance of microorganisms to antibiotics and the cost of modern allopathic medicines, the scientists are now looking for medicinal plants, because most of them are safe, cost less and are effective against a wide range of antibiotic resistant microorganisms. In this context, the present study elucidates the

***Corresponding Author Address:** Dr. A.Sivaraj, Ph.D. Young Scientist – SERB, Department of Zoology, Thiruvalluvar University, Serkkadu, Vellore- 632 115, Tamilnadu, India; E. mail: sivaraj80_cahc@yahoo.co.in*

antimicrobial activity of aqueous extract of *Eucalyptus oblique* against selective *Vibrio* spp.

## MATERIALS AND METHODS

***Plant:*** The leaves of *Eucalyptus oblique* (Family: Myrtaceae) were collected during the month of November 2015 from in and around Vellore District, Tamilnadu, India. The plant materials were cleaned with distilled water and shade dried at room temperature. The plant material was authenticated and voucher specimens were kept at the Department of Zoology, Thiruvalluvar University, Vellore Dt., Tamilnadu. The shade dried plant materials were powdered by using electric blender.

***Preparation of plant extract:*** The powdered plant materials were extracted separately to exhaustion in a soxhlet apparatus of aqueous plant extracts (Merk Chemicals, India). The extract was filtered through a cotton plug followed by Whatman filter paper (No.1) and then concentrated by using a rotary evaporator at low temperature (40-50°C) and reduced pressure to get yield. The extract was preserved in airtight containers and kept at 4°C until further use. The aqueous crude extract was taken for *invitro* antibacterial studies.

***Primary bacterial culture:*** Water samples from the *P. monodon* hatchery culture tank were taken in a culture flask to identify the presence of *Vibrio* spp. Appropriate dilutions were made in sterile PBS, and 100-ml samples were plated on TCBS agar. Nine colonies from different plates, displaying typical morphology on TCBS, were isolated in pure culture and presumptively identified to the species level by the method of Alsina and Blanch.

***Maintenance of test bacterial strains:*** All the microorganisms used in the study were maintained on Nutrient agar (Himedia, Mumbai) slants and kept in refrigerator; sub-cultures were made after every fifteen days.

***Antibacterial activity (Agar well diffusion method):*** The extract obtained from plant material was studied for antimicrobial activity. The water sample from *P.monodon* hatchery was inoculated in 100 ml of nutrient broth in a conical flask and incubated for 24 hrs to activate the strain. 100µl culture inoculums ($1x10^6$cfu/ml) from nutrient broth was inoculated into sterile TCBS agar plates for 48hrs. Different colonies of *Vibrio* spp. formed in as the TCBS agar plates. The colonies were identified based on the size and colour of the specific species. For pure culture, a loop full of specific colony was inoculated in the Muller Hinton Broth for 24 hours. 100µl of pure culture was inoculated into solidified Muller Hinton agar. Care was taken to ensure proper homogenization. The experiment was performed under strict aseptic conditions. A well was made in the plates with sterile borer (5mm). The extract compound (50 µl) was introduced into the well and the plates were incubated at 37 ˚C for 24 to 48 hrs. All samples were tested in triplicates. Microbial growth was determined by measuring the diameter of the zone of inhibition. Ciprofloxacin (Himedia, Mumbai, India) is a reference drug used as a control for test organisms.

***Determination of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC):*** The plant extract which was found to be effective as antimicrobial agent, was later tested to determine the MIC and MBC values for selected strain. MIC was determined using broth dilution method. The plant extract was diluted with 5ml Muller Hinton broth to give the final concentrations of 1000, 500, 250, 125, 62.5, 31.25µg/ml. The different concentration of plant extract was taken in six separate MIC tubes and 100 µl ($10^6$ CFU/ml) of the pure *Vibrio* spp. from the TCBS agar was inoculated into the tubes. The tubes were incubated under aseptic condition at 37°C for 24 hrs. Streptomycin which was used as the positive control (15µg) was added into 5 ml of Muller Hinton broth taken in the control tube. The lowest concentration of the extract that produced no visible growth (no turbidity) in the first 24 h when compared with the control tubes was considered as initial MIC. The dilutions that showed no turbidity were incubated further for 24 h at 37 °C. The lowest concentration that produced no visible turbidity after a total incubation period of 48 h was regarded as final MIC.

The minimum bactericidal concentration (MBC) was defined as the minimal concentration of the plant extract which completely inhibited the visible growth of the bacteria on solid media in the petriplates that were incubated at 37ºC for 24 hrs.

## RESULTS

***Agar well diffusion method:*** In the present study the antibacterial activity of *Eucalyptus oblique* aqueous leaf extract was evaluated against seven *Vibrio* spp. (Table:2). The aqueous leaf extract of *Eucalyptus oblique* showed more antibacterial activity against all *Vibrio* species like *Vibrio harveyi*, *Vibrio natriegens*, *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, *Vibrio fluvialis*, *Vibrio anguillarum* and *Vibrio vulnificus.* The zone inhibitory activity of plant extract reported in (Table: 2) was compared with standard reference antibiotic Ciprofloxacin (15µg).

***Minimum inhibitory concentration (MIC):*** The MIC value of *Eucalyptus oblique* aqueous leaf extract against Vibrio spp. is shown in table 3. Generally, if the extract displayed an MIC in well diluted form (e.g. below 100 µg/ml), then its antimicrobial activity was considered to be the best; if in moderate dilution (e.g., from 100 to 500 µg/ml) the antimicrobial activity was considered to be better; and if in more concentration during dilution (e.g., from 500 to 1000 µg/ ml) the antimicrobial activity was considered as good. If the same was above 1000 µg/ml then the extract was considered inactive. The leaf extract leaf extract of *Eucalyptus oblique* presented best activity against *Vibrio* Spp. like *Vibrio harveyi* (MIC 62.5), *Vibrio natriegens* (MIC 31.25), *Vibrio alginolyticus* (MIC 31.25), *Vibrio parahaemolyticus* (MIC 62.5), *Vibrio fluvialis* (MIC 125), *Vibrio anguillarum* (MIC 125) and *Vibrio vulnificus* (MIC 31.25) (Table : 3).

***Minimum bactericidal concentration (MBC):*** The results for Minimum Bactericidal Concentration (MBC) were similar to Minimum Inhibitory Concentration (MIC) results, but in MBC confirmation was made by absence of growth in culture plates. It is expressed in table 3.

## DISCUSSION

The medicinal plants constitute an effective source of both traditional and modern medicines. The present study was conducted to investigate the *invitro* antibactetial activity of aqueous leaf extract of *Eucalyptus oblique* against *Vibrio* spp of *P.monodon* hatchery. The plant extract showed high antibacterial activity against *Vibrio vulnificus*, *Vibrio alginolyticus* and *Vibrio natriegens* (Table:2,3). The strong antibacterial activity of plant extract might be due to presence of various phytochemical constituents, such as alkaloids, tannins, saponins, cardenolides, flavonoids and polyphenols. Phenolic compounds are generally noted for their antimicrobial activities [9]. These phytochemical constituents may be present in the *Eucalyptus oblique* extract which is responsible for its antibacterial effect.

The *Eucalyptus oblique* extract can be used as an alternative therapeutic agent to prevent and control the outbreak of diseases, mainly in hatcheries. Since the plant substance is natural, their hazardous potential is lower when compared with other products. The results proved that the *Eucalyptus oblique* plant has a high potential for use as an alternative therapy to control bacterial of crustacean diseases [10]. This is to be expected because the outer membrane of the bacteria is known to be a barrier for the penetration of numerous antibiotic molecules, and the periplasmic space contains enzymes, which are capable of breaking down foreign molecules introduced from outside [11]. It was interesting to note that the extract as able to control *Vibrio* species. It has been concluded form this study that *Eucalyptus oblique*, can be used as a potent antibacterial drug in the crustacean aquaculture instead of synthetic antibiotics.

***Acknowledgement:*** The authors express their sincere thanks to the Science and Engineering Research Board, New Delhi, India for providing financial support. The authors also thankful to the Thiruvalluvar University, Serkkadu, Vellore -632 115, Tamilnadu, India for providing facilities to complete this work.

**Table 1: Colony morphologies on TCBS agar**

| Species | Colony morphology |
|---|---|
| ***Vibrio harveyi*** | Small (diameter, 2 to 5 mm) colonies; light green with dark |
| ***Vibrio natriegens*** | Small (diameter, 2 to 5 mm) colonies; light green |
| ***Vibrio alginolyticus*** | Large (diameter, 5 mm) colonies, spreading; light blue |
| ***Vibrio parahaemolyticus*** | Large (diameter, 5 mm) colonies, spreading; light blue |
| ***Vibrio fluvialis*** | Small (diameter, 2 to 5 mm); dark blue-green |
| ***Vibrio anguillarum*** | Very small (diameter, 2 mm); light blue |
| ***Vibrio vulnificus*** | Small (Diameter 2 to 5mm) colonies; light green |

**Table 2: Antibacterial activity of aqueous leaf extract of *Eucalyptus oblique* against *Vibrio* Spp. of *P.monodon* hatchery**

| ***Vibrio* Spp.** | **Zone of Inhibition (in mm)** | **Ref Drug Ciprofloxacin (15µg)** |
|---|---|---|
| *Vibrio harveyi* | 10.2 ± 0.30 | 14.5 ± 0.15 |
| *Vibrio natriegens* | 9.9 ± 0.10 | 14.5 ± 0.15 |
| *Vibrio alginolyticus* | 11.1 ±0.32 | 14.5 ± 0.15 |
| *Vibrio parahaemolyticus* | 10.3.± 0.15 | 14.5 ± 0.15 |
| *Vibrio fluvialis* | 9.6 ± 0.32 | 14.5 ± 0.15 |
| *Vibrio anguillarum* | 9.8± 0.12 | 14.5 ± 0.15 |
| *Vibrio vulnificus* | 10.3 ± 0.26 | 14.5 ± 0.15 |

**Table 3: Determination of MIC and MBC of aqueous leaf extract of *Eucalyptus oblique***

| **Vibrio Spp.** | **MIC (µg/ml)** | **MBC (µg/ml)** | **Ref Drug** Ciprofloxacin (15µg) |
|---|---|---|---|
| *Vibrio harveyi* | 62.5 | 62.5 | 15 |
| *Vibrio natriegens* | 31.25 | 31.25 | 15 |
| *Vibrio alginolyticus* | 31.25 | 31.5 | 15 |
| *Vibrio parahaemolyticus* | 62.5 | 62.5 | 15 |
| *Vibrio fluvialis* | 125 | 125 | 15 |
| *Vibrio anguillarum* | 125 | 125 | 15 |
| *Vibrio vulnificus* | 31.25 | 31.25 | 15 |

## REFERENCES


1. Klinger D. H. *et al.* Moving beyond the fished or farmed dichotomy. Marine Policy. 2012.
2. Anon. Handbook on fisheries statistics. Ministry of Agriculture (Department of Agriculture and Co-operation, Fisheries Division), Government of India. 2000; 153.
3. Katiha P.K. et al. Profile of key aquacultural technologies in India. In: A profile of People, Technologies and Policies in Fisheries Sector of India, pp 59–82. National Centre for Agricultural Economics and Policy Research, New Delhi. 2003.
4. Chen F.R. Lethal attribute of serine protease secreted by *Vibrio alginolyticus* strains in Kurama Prawn *Penaeus japonicus*. *Zool Natur. forsch*. 2000; 55: 94-99.
5. Bergh O. *et al.* Diseases, prophylaxis and treatment of the Atlantic halibut *Hippoglossus hippoglossus*: a review. *Dis Aquat Org.* 2001; 48:57-74.
6. Direkbusarakom S. Application of medicinal herbs to aquaculture in Asia. *Walailak J. Sci. Technol*. 2004;1(1):7-14.
7. Abutbul, S. *et al.* Screening of desert plants for use against bacterial pathogens in fish. Isr *J. Aquaculture Bamid* 2005; 57(2):71-80.
8. Rios J.l, and Recio M.C. Medincinal plants and antimicrobialactivity. *J.Ethnopharmacol*. 2005; 100:80-84.
9. Evans J.R. *Escherichia coli.* Medical microbiology. 4th Edn. The University of Texas Medical Branch at Galveston. 2007.
10. Castro, S.B.R. *et al.*. Antibacterial activity of plant extracts from Brazil against fish pathogenic bacteria. *Braz. J Microbiol.* 2008; 39(4):756-760.
11. Duffy C.F. and Power R.F. Antioxidant and antimicrobial properties of some Chinese plants extracts. *Int. J. Antimicrob. Agents.* 2001; 17: 527-529.