INVITRO ANTIVIBRIO ACTIVITY OF *Embilica officinalis* AND *Ocimum sanctum* AGAINST *Vibrio* sp ISOLATED FROM DISEASED *Penaeus monodon* (Fab).

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ABSTRACT

The biggest problem faced by the aquaculture industry in world wide is diseases caused due to various biological and non biological agents. Among the two plants tested the alcoholic extract of *Ocimum sanctum* showed maximum zone of inhibition against *Vibrio cholerae* and *Vibrio parahaemolyticus* (14±2.6mm).

Key words: *Ocimum*, Alcoholic extract, Shrimp, *Penaeus* sp, Inhibition.

INTRODUCTION

Aquaculture is gaining significant momentum in several parts of India. India is one of the leading countries of aquaculture practicing mainly shrimp production and its export since flesh consist of rich proteins shrimps are cultured in water rich in natural feed, which is derived from both plants like phytoplankton, algae and animals like zooplankton and benthic animals. Supplementary feed are a source of nutrients, which complement natural food to increase shrimp production capacity these low cost feeds are processed pellets which provide primary protein and energy to the culture systems. Asia is the hub of aquaculture, industries contributing 80% of the world’s aquaculture production. Aquaculture has been a tradition in several parts of Asia and according to FAO statistics over 80% of fish produced by aquaculture come from Asia. Aquaculture is defined as the husbanding of aquatic organism under the controlled condition.

The tiger shrimp *Penaeus monodon* is the fast growing species. It grows to about 12 cm with average weight of 30 g in three months and 22 cm and average weight of 95 g in one year the shrimp *Penaeus monodon* the largest among the marine prawns growing to about 32 cm. They feed throughout the day they consume more at night than during the day. The food intake increase during the withraise of water temperature. Thus shrimp consume more food during the
warmer months than during the colder month. The shrimp *Penaeus monodon* breed in the surface or subsurface water of the sea near the estuary. The life history of *Penaeus monodon* spent partly in estuaries or brackish water during early juvenile phase and later in the sea.

The prawn fishery is the most important fishery which earns large amount of foreign exchange. The shrimps one of the most esteemed food among the marine organism. Numerous varieties of prawns and shrimps contribute the fishery of in India. These species can be cultured in polyculture and monoculture system. They attain the marketable size above 47g with 6 months with a survival rate of 90-95 percent depending on the size of juveniles stocked. They are continuous breeders with two breeding peaks in a year. One peak breeding seasons is in October-November and the second one in May and June.

The biggest problem faced by the aquaculture industry worldwide is diseases caused due to various biological and non-biological agents. Among the groups of microorganisms that cause serious losses in shrimp culture, the best known are bacteria because of the devastating economic effects they have on affected forms. Microbes mainly cause the diseases. Microorganisms are ubiquitous in nature extremely diverse group and are microscopic in nature which includes viruses, bacteria, fungi, Protozoa and some algae. Fish diseases are one of the major problems in the fish farm industry. Even through vaccines are being developed and marketed they cannot be used as universal disease control measure in aquaculture. The shrimp industry is facing serious problem with several types of viral diseases. Nearly 10-12 kinds of viruses are responsible for causing diseases baculoviral, midout gland, necrosis, white spot syndrome, yellow head disease.

Bacterial diseases particularly Vibriosis causes high mortality rate in aquaculture. Vibriosis one of the major disease problems in shell fish and fin fish aquaculture. Vibriosis is a bacterial disease responsible for mortality of cultured shrimp worldwide (Lightner and Lewis, 1975). Vibriosis is caused by a number of *Vibrio* sp of bacteria including *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio harveyi* (L) *Vibrio harveyi* (NL).

*Vibrio parahaemolyticus* is also known to cause sea food- borne infections such as septicemia and wound infections has been reported to be responsible for 95% of sea food related deaths. Extra intestinal *Vibrio* infections often result in serious disability or death, (Whitman
and griffin, 1993). *Vibrio parahaemolyticus* is gram negative, non-spore forming bacterium normally found in marine environments. It is the most likely *Vibrio* species to be implicated in food borne diseases *Vibrio parahaemolyticus* is found mainly in foods of marine origin and studies carried out in the United State found that 60 to 100% of sea food samples were contaminated with the organisms. *Vibrio harveyi* a gram – negative luminous bacterium is one of the important ethologic agents of mass mortalities of *Penaeus monodon* larval rearing systems. Among the *Vibrio harveyi* isolates, some are virulent and some are not, suggesting a great deal of molecular and genetic variation in this group of bacteria. Luminescent *Vibrio harveyi* appears to release exotoxins (Liu et al., 1996), and may cause 80 to 100% mortality in *Penaeus monodon* hatcheries (Harris, 1995). Bacterial diseases in aquaculture are mainly controlled by antibiotics however resistance and there by to a reduced efficacy of the drugs. In the public health context, antibiotic resistance can be transferred to environmental and human pathogenic bacteria (Alderman and Hastings., 1998).

Traditional medicines represented mainly by plants have becomes an alternatives as they are considered synthetic antibiotics. Hence, the need to increase the relatively safer and more affordable when compared to body of knowledge on the antimicrobial activities of some traditional medicinal plants towards curbing the effects of antibiotic resistance in *Vibrio* species become imperative. Species and herbs have been used for thousands of centuries by many cultures to enhance the flavor and aroma of foods. Early cultures also recognized the value of using species and herbs in preserving foods and for their medicinal value. Scientific experiments since the late 19th century have documented the antimicrobial properties of some species, herbs and their components. Several of the species and their essential oil reported to passes antimicrobial activities including *Embilica officinalis* and *Ocimum sanctum* (Arora and Kaur, 1999).

*Embilica officinalis* (Gaertn), a medicinal plant native of India called as ‘Nellikai’ is extensively used in Ayurvedic and Unani Systems of medicine (Kritikar and Basu 1975). It has anti-viral, antibacterial, anti cancer, anti allergic and anti-mutagenic properties (Nair et al., 2007). The major principles in *Embilica officinalis* active against microbes, include, flavonoids (querction), ascorbic acid, gallic acid, alkaloids (phyllantine, phyllantidine) and hydrolysable tannins (emblicanin A and B) (Patil et al., 2012). Fruits of *E.officinalis* are the richest source of
vitamin C, tannin and flavonoids, most of which are antioxidant in action invitro antibacterial activity of fruits, seeds, stem, leaf, and extracts of *E.officinalis* against eight species of pathogenic bacteria.

The *Ocimum sanctum* the tamil name of the plant is dulasi is a herbal plant having antimicrobial activity against many of the microorganism and also has the anticancer, antidiabetic (Mediratte and Sharma 2000). A variety of biologically active compounds such as ursolic acid, apigenin and luteolin have been isolated from the leaves. Phytochemical compounds in leaf include eugenol (volatile oil) ursolic acid (triterpenoid) and rosmarinic acid ( phenylpropanoid) other active compounds include caryphyllene and oleanolic acid. Nutritional components include vitamin A and C, Minerals, Calcium, Iron and Zinc as well as chlorophyll. Tannins, alkaloids, glycosides and saponins are abundant in tulsi. Antibacterial activity with increase in concentration and specified contact time (SadulRama et al., 2009). However to date there have been scientific investigations are need to determine the safety and efficacy of plant- based medicines as feed additives. Hence an attempt has been made to screen the antivibrio activity of selected *Vibrio* sp isolated from diseased Shrimp *P. monodon* against selected medicinal plants such as, *Embilica officinalis* and *Osimum sanctum*. And To Compare the efficacy of antivibrio properties of selected medicinal plants


Chandrakala et al.,(2013 a) studied the preliminary screening of *Curcuma longa* (linn) extract against *Penaeus monodon* (Fab) pathogenic Vibrio sp. Varghese et al .,(2013) Made a comparative antibacterial activity of fruit extract of *Embilica officinalis* (gaertn) against gram positive and gram negative bacteria Chandrakala et al.,(2013 b) studied the Gas chromatography mass spectrum analysis and antivibrio activity of *Andrographis paniculata*. Amin Mir et al., (2013) studied the qualitative and quantitative analysis of *Taxaxcum officinalis*. Varghese et al., (2013) studied the evaluation of invitro antibacterial activity whole plant fruits, seeds, stem, leaves and root of *Embilica officinalis gaertn*. Chandrakala et al., (2013) studied the screening of bioactive compounds and testing the antagonistic effect of *Zinger Officinalis (linn)* against *Penaeus monodon* pathogenic Vibrio sp. Harikrishna et al., (2014) studied the antibacterial potential and corrosion inhibition efficiency of *Embilica officinalis* (Amla).

**MATERIALS AND METHODS**

Experiment plants such as *Embilica officinalis* (Geartn) and *Ocimum sanctum* (Linn), were freshly collected from Gopanlugar, Marungulam, Thanjavur (Dt), Tamilnadu (10.46°N;79.10° E). The pathogenic bacterial species such as, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio harveyi* (L), *Vibrio harveyi* (NL), were selected and were isolated from the diseased *Penaeus monodon* (Chandrakala and Ayyavoo, 2006). About one gram of sterilized plant leaves were grind in motar and pestle with 50ml aqueous and organic solvent (Ethonol). It was filtered and the supernatant was stored for antibacterial study (Parihar and Bhora 2002).

The antibacterial activity of the leaves were tested against the selected bacterial species (Mohney et al.,1992) after sterilization TCBS agar medium was poured into petriplates and allowed the solidify. Swab was made with test culture (10 ^4 cfu/ml:18hrs) and 5mm size well was made in the agar plates. The wells were loaded with extract and the solvent loaded into well
without extracts served as control. The antibacterial activity of plant extract was compared with commonly used antibiotics. All the plates were incubated at 37° for 24hrs. After incubation the plates were observed for formation of inhibition zone around the well.

RESULTS

The antivibrio activity of selected medicinal plants such as *Embilica officinalis* and *Ocimum sanctum* revealed the following observation. In *Embilica officinalis* the water extract the zone of inhibition was observed only in *Vibrio cholerae* (10±1.7) and no zone was observed in other test organism. Among the alcohol extract revealed that the maximum zone was observed in *Vibrio harveyi* (L) (13±2.4) and was followed by the *Vibrio paraahaemolyticus* (12±2.3) and *Vibrio harveyi* (NL) (12±2.3) and was followed by the *Vibrio cholerae* (11±2.0). In *Ocimum sanctum* the water extract showed the maximum zone of inhibition against *Vibrio cholerae* (12±2.3) and was followed by *Vibrio paraahaemolyticus* (10±1.7) and *Vibrio harveyi* (NL) (10±1.7) and no zone was observed in *Vibrio harveyi* (L). Among the alcohol extract revealed that the maximum zone was observed in *Vibrio cholera* (14±2.6) and *Vibrio paraahaemolyticus* (14±2.6) and was followed by other test organism *Vibrio harveyi* (L) (12±2.3) and *Vibrio harveyi* (NL) (12±2.3). Among the two plants tested against *Vibrio species* the alcohol extract of *Ocimum sanctum* showed maximum zone of inhibition against *Vibrio cholerae* and *Vibrio paraheamolyticus* (14±2.6). Among the water extract test plants *Ocimum sanctum* showed maximum zone (12±2.3) of inhibition against *Vibrio cholerae* (Table 1-3). Hence the present study revealed that the water and alcohol extract of *Ocimum sanctum* showed the maximum zone of inhibition and in feature the study may be taken up with the medicinal plants used as formulated feed to reduce the disease outbreak.

DISCUSSION

Medicinal plants are one of the most commonly used natural antimicrobial agents in food and have been used traditionally for thousands of years by many cultures for controlling common health complications and antimicrobial potency of plants are believed to be due to tannins, saponins phenolic compounds, essential oils and flavonoids, (Aboaba and Efuwape., 2001). Plants certain numerous biologically active compounds many of which have been shown to have antimicrobial properties (Menghai *et al.*, 2011).
Park et al. (2008) observed maximum zone of inhibition against E.coli and P.aeruginosa. Kamalesh et al. (2013). The methanolic extract effectively controlled the pathogens such as, Salmonella Vibrio spp, Versina and Aermonas spp in the F.indicus. Invitro herbal diets prepared from the five herbs such as, Adathoda vosica, Murraya koenijii, Ocimum bacilium, Psoralea corylifolia, and Quercus infectoria were effectively suprressed the pathogens such as, Pseudomonas aeruginosa, Staphylococcus aureus, Aeromonas hydrophilia, Vibrio harveyi and Vibrio parahaemolyticus in the P.monodon immune system. The essential oil have been shown to exhibit high antibacterial activity against Staphylococcus areus, E.coli, Bacillus substiliius.

Chandrakala et al. (2013 a) observed that the ethanolic extract of turmeric Curcuma longa which contained 20.0% by dry weight of Curcumin showed inhibitory effect against the six species of Vibrio including Vibrio harveyi, Vibrio cholerae, Vibrio parahaemolyticus, V.alginalyticus, V.vulnificus. The minimum inhibitory effect was observed with ethanolic extract on V.harveyi, V.cholerae, V.parahaemolyticus, V.alginolyticus, V.vulnificus and V.fluvialis at concentration of 0.47, 0.47, 0.94, 0.47, 3.75, and 0.47mg/disk respectively.

Chandrakala et al. (2013 b) reported that the Vibrio sp isolated from Penaeus monodon, Sillago shigama and canned sea food revealed the presence of V.cholerae, V.parahaemolyticus, V.harveyi the antivibrio activity of selected medicinal plants revealed cumen showed maximum zone of inhibition.

In the present study on invitro screening of the medicinal plants against shrimp pathogenic Vibrio revealed that among the two plants tested against Vibrio spp. the alcohol extract of Ocimum sanctum showed maximum zone (14±2.6) of inhibition against Vibrio cholerae and Vibrio parahaemolyticus. Among the water extract the test plants Ocimum santum showed maximum zone (12±2.3) of inhibition against Vibrio cholerae.

Shatyajit et al. (2012) study the antibacterial activity of E.officinalis fruit extract was evaluated against four pathogenic bacteria belonging to both gram positive and gram negative group. Extract of E.officinalis acetone fruit extract has the maximum antibacterial activity against E.coli than by other extract against other test organism. Varghese et al. (2013) in the study of the aqueous extract of Embilica exhibited fairly good antibacterial activity Klebisella
which could be effectively exploited in the treatment of *Pneumonia* in *Tuber culosis* patients. Mann *et al.*, (2000) reported that the essential oils of *Ocimum* had highest antibacterial activity against gram positive bacteria as opposed to gram negative bacteria.

**TABLE.1**

INHIBITION ZONE OF *Embilica Officinalis* AGAINST *Vibrio* sp.

<table>
<thead>
<tr>
<th>TEST ORGANISM</th>
<th>ALCOHOLIC EXTRACT (mm)</th>
<th>WATER EXTRACT (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vibrio cholera</em></td>
<td>11±2.0</td>
<td>10±1.7</td>
</tr>
<tr>
<td><em>Vibrio paraheamolyticus</em></td>
<td>12±2.3</td>
<td>_</td>
</tr>
<tr>
<td><em>Vibrio harveyi(L)</em></td>
<td>13±2.4</td>
<td>_</td>
</tr>
<tr>
<td><em>Vibrio harveyi(NL)</em></td>
<td>12±2.3</td>
<td>_</td>
</tr>
</tbody>
</table>

**TABLE.2**

INHIBITION ZONE OF *Ocimum sanctum*, AGAINST *Vibrio* sp.

<table>
<thead>
<tr>
<th>TEST ORGANISM</th>
<th>ALCOHOLIC EXTRACT (mm)</th>
<th>WATER EXTRACT (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vibrio cholera</em></td>
<td>14±2.6</td>
<td>12±2.3</td>
</tr>
<tr>
<td><em>Vibrio paraheamolyticus</em></td>
<td>14±2.6</td>
<td>10±1.7</td>
</tr>
<tr>
<td><em>Vibrio harveyi(L)</em></td>
<td>12±2.3</td>
<td>_</td>
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</tbody>
</table>
TABLE 3

INHIBITION ZONE OF SELECTED MEDICINAL PLANT EXTRACTS AGAINST Vibrio sp

<table>
<thead>
<tr>
<th>TEST ORGANISM</th>
<th>Embilica officinalis</th>
<th>Ocimum sanctum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alcohol (mm)</td>
<td>Water (mm)</td>
</tr>
<tr>
<td>Vibrio cholera</td>
<td>11±2.0</td>
<td>10±1.7</td>
</tr>
<tr>
<td>Vibrio paraheamolyticus</td>
<td>12±2.3</td>
<td>-</td>
</tr>
<tr>
<td>Vibrio harveyi (L)</td>
<td>13±2.4</td>
<td>-</td>
</tr>
<tr>
<td>Vibrio harveyi (NL)</td>
<td>12±2.3</td>
<td>-</td>
</tr>
</tbody>
</table>

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