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Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

COMPARATIVE STUDY ON THE *INVITRO* ANTIBACTERIAL ACTIVITY OF SELECTED MEDICINAL PLANTS AGAINST PATHOGENIC *VIBRIO* SPECIES FROM DISEASED *PENAEUS MONODON* (FAB)

N. Chandrakala*, P. Parameswari

PG & Research Department of Zoology, Kundavai Naacchiyaar Govt Arts College (W) Autonomous, (Affiliated to Bharathidasan University), Thanjavur, Tamilnadu, India

**Corresponding author:dr.n.chandrakala@kngac.ac.in*

ABSTRACT

The pathogenic *Vibrio sp.* were isolated from diseased shrimp of *Penaeus monodon* (FAB) using TCBS agar medium by streak plate method. *Acalipha indica* (L), *Vinca rosea* (L), *Embilica officinalis* (G), *Ocimum sanctum*(L) and *Lucas aspera* (L) medicinal plants are one of the most commonly used natural antimicrobial agents used traditionally. Hence an attempt has been made to screen the antivibrio activity of some selected medicinal plants against pathogenic *Vibrio sp* by disk diffusion method. Among the five plants tested, water extract of *Lucas aspera* showed the maximum zone of inhibition against *Vibrio cholerae* (18±3.4 mm in diameter) and the alcohol extract showed the maximum zone of inhibition against *Vibrio harveyi (NL)* (16±3 mm in diameter).

Keywords:*Penaeus* sp, *Invitro*, Antibacterial Activity, Luminescence (L), Non Luminescence (NL).

1. INTRODUCTION

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. 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 fed. It is the world's fastest growing food production with the cultured shrimp and prawn subsector growing at an annual rate of 16.8% between 1984 and 1995 [1]. The impact of diseases in aquaculture is enormous and millions of dollars is lost annually worldwide. Shell fish are important sea food for human consumption. The prawn fishery is most important which earns large amount of foreign exchange. 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. Bacterial diseases particularly Vibriosis causes high mortality rate in aquaculture. Vibriosis is one of the major disease problems in shell fish and fin fish aquaculture [2]. *Vibrosis* is caused by a number of *Vibrio* species of bacteria including *Vibrio cholera, Vibrio parahaemolyticus, Vibrio harveyi (L)* and *Vibrio harveyi (NL)*. Among the major diseases caused by *Vibrio* Species is cholera which occurs when *Vibrio cholera* colonizes in the small intestine and releases on enterotoxin [3]. *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 [4]. Luminescent *Vibrio harveyi* appears to release exotoxins [5], and may cause 80 to 100% mortality in *Penaeus monodon* hatcheries [6].

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 medicine 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. In recent years, herbal plant supplements have been developed as an innovation and could provide a reliable long term answer for the industry facing the antibiotics ban. The medicinal herbs contain physiologically active principle that over the years exploited in traditional medicine for the treatment of various oilments as they have anti- microbial properties [7]. Several of the species and their essential oil reported to possess antimicrobial activities including *Acalipha*

indica, *Vinca rosea*, *Embilica officinalis*, *Ocimum sanctum* and *Lucas aspera* [8]. Hence an attempt has been made to screen the antivibrio activity of selected *Vibrio* species isolated from diseased shrimp *P. monodon* against selected medicinal plants such as, *Acalipha indica, Vinca rosea, Embilica officinalis, Osimum sanctum,* and *Lucas aspera* to compare the efficacy of *antivibrio* properties of selected medicinal plants.

2. MATERIAL AND METHODS

Experiment plants such as *Acalipha indica (L), Vinca rosea (L), Embilica officinalis (G), Ocimum sanctum(L)* and *Lucas aspera (L)* were freshly collected from Gopalnagar, Marungulam, Thanjavur (Dt), Tamilnadu (10.46°N; 79.10°E). The pathogenic bacteria species such as *Vibrio cholera, Vibrio parahaemolyticus, Vibrio harveyi (L)* and *Vibrio harveyi (NL)* were selected and were isolated from the diseased *Penaeus monodon* [9]. About one gram of sterilized plant leaves were ground in motar and pestle with 50ml aqueous and organic solvent (Ethonol). It was filtered and the supernatant was stored for antibacterial study [10]. The antibacterial activities of the leaves were tested against the selected bacterial species [11]. TCBS agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45°C. The cooled media was poured on to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab (10^4cfu/ml/18 Hrs). 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°C for 24Hrs. After incubation, the plates were observed for formation of inhibition zone around the well.

3. RESULTS AND 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 [12]. Plants contain numerous biologically active compounds many of which have been shown to have antimicrobial properties [13].

The antivibrio activity of selected medicinal plants such as *Acalipha indica, Vinca rosea, Embilica officinalis, Ocimum sanctum* and *Lucas aspera* revealed the following observation. In *Acalipha indica* the water extract showed the maximum zone of inhibition against *Vibrio cholera* (13±2.4 mm in diameter) and was followed by *Vibrio parahaemolyticus* (10±1.7 mm in diameter) and *Vibrio harveyi* (L) (10±1.7 mm in diameter) and no zone was observed in *Vibrio harveyi* (NL). But the alcohol extract revealed that maximum zone was observed in *Vibrio harveyi (L)* (13±2.4 mm in diameter) and was followed by other test organism (Table 1).

Table 1:Inhibition zone of *Acalipha Indica* against *Vibrio* species

Test Organism	Zone of Inhibiton (mm in Diameter)	
	Alcoholic Extract	Water Extract
Vibrio cholera	10±1.7	13±2.4
Vibrio parahaemolyticus	10±1.7	10±1.7
Vibrio harveyi (L)	13±2.4	10±1.7
Vibrio harveyi (NL)	10±1.7	-

L - Luminescence strain; NL - Non Luminescence strain, Values are expressed Mean ± Standard Deviation; n=3

The methanolic extract effectively controlled the pathogens such as *Salmonella vibrio sp., Versina* and *Aermonas sp.* in the *F. indicus*. *In vitro* herbal diets prepared from the five herbs such as, *Adathoda vosica, Murraya koenijii, Ocimum bacilium, Psoralea corylifolia* and *Qurcusin fectoria* effectively suppressed the pathogens such as, *Pseudomonas aeruginosa*, *Staphylococcus aureus, Aeromonas hydrophilia*, *Vibrio harveyi* and *Vibrio parahaemolyticus* in the *P. monodon* immune system. The essential oil has been shown to exhibit high antibacterial activity against *Staphylococcus areus, Escherichia coli,* and *Bacillus subtilis*.

In *Vinca rosea* water extract, the zone of inhibition was observed only in *Vibrio cholera* (12±2.3 mm in diamter) and no zone was observed for other test organism. But the alcohol extract revealed the maximum zone of inhibition against *Vibrio harveyi* (NL) (15±2.8 mm in diameter) and was followed by *Vibrio harveyi* (L) (14±2.6 mm in diameter) and *Vibrio cholerae* (10±1.7 mm in diameter) and *Vibrio parahaemolyticus* (10±1.7 mm in diameter) (Table 2).

Similarly Naz et al. [14] evaluated antimicrobial activity of Vinca rosea against pathogenic bacterial and fungal strains by agar well diffusion method. Ethanolic extracts showed better results as compared to the *in vivo* extracts for both the antibacterial as well as the antifungal activity. The antivibrio activity of *Vinca rosea* medicinal plants revealed maximum zone of inhibition possess a

great strength to fight against the microbial activity and can be used against various infections [15].

In *Embilica officinalis* water extract, the zone of inhibition was observed only in *Vibrio cholera* (10±1.7 mm in diameter) and no zone was observed in other test organism. But the alcohol extract revealed that the maximum zone was observed in *Vibrio harveyi* (L) (13±2.4 mm in diameter) and was followed by the *Vibrio parahaemolyticus* (12±2.3 mm in diameter) and *Vibrio harveyi* (NL) (12±2.3 mm in diameter) and was followed by the *Vibrio cholera* (11±2.0 mm in diameter) (Table 3). In this study *Vibrio cholera* only inhibited differently reported *Embilica officinalis* aqueous fruit extracts revealed potent antibacterial effects for five human bacterial pathogen stock cultures, namely *Bacillus sp., Lactobacillus sp., Pseudomonas sp., Proteus sp., Streptococcus* species [16].

Table 2:Inhibition zone of *Vinca rosea* against *Vibrio* Species

Test Organism	Zone of Inhibition (mm in Diameter)	
	Alcoholic Extract	Water Extract
Vibrio cholera	10±1.7	12±2.3
Vibrio parahaemolyticus	10±1.7	-
Vibrio harveyi (L)	14±2.6	-
Vibrio harveyi (NL)	15±2.8	-

L - Luminescence strain; NL - Non Luminescence strain. Values are expressed Mean ± Standard Deviation; n=3

Table 3:Inhibition zone of *Embilica officinalis* against *Vibrio* species

Test Organism	Zone of Inhibition (mm in Diameter)	
	Alcoholic Extract	Water Extract
Vibrio cholera	11±2.0	10±1.7
Vibrio parahaemolyticus	12±2.3	-
Vibrio harveyi(L)	13±2.4	-
Vibrio harveyi(NL)	12±2.3	_

L - Luminescence strain; NL - Non Luminescence strain, Values are expressed Mean ± Standard Deviation; n=3

In *Ocimum sanctum,* the water extract showed the maximum zone of inhibition against *Vibrio cholera* (12±2.3 mm in diameter) and was followed by *Vibrio parahaemolyticus* (10±1.7 mm in diameter) and *Vibrio harveyi* (NL) (10±1.7 mm in diameter) and no zone was observed in *Vibrio harveyi* (L). But the alcohol extract revealed that the maximum zone was observed in *Vibrio cholera* (14±2.6 mm in diameter) and *Vibrio parahaemolyticus* (14±2.6 mm in diameter) and was followed by other test organism *Vibrio harveyi* (L) (12±2.3 mm in diameter) and *Vibrio harveyi* (NL) (12±2.3 mm in diameter) (Fig. 1).

In *Lucas aspera,* the water extract showed the maximum zone of inhibition against *Vibrio cholera* (18±3.4 mm in diameter) followed by *Vibrio harveyi* (NL) (12±2.3 mm in diameter), *Vibrio parahaemolyticus* (11±2.0 mm in diameter) and *Vibrio harveyi* (L) (10±1.7 mm in diameter). But the alcohol extract revealed that maximum zone of inhibition was observed in *Vibrio harveyi* (NL) (16±3 mm in diameter) and was followed by *Vibrio parahaemolyticus* (14±2.6 mm in diameter), *Vibrio harveyi* (L) (13±2.4 mm in diameter) and *Vibrio cholera* (11±2.0 mm in diameter) (Fig. 2).

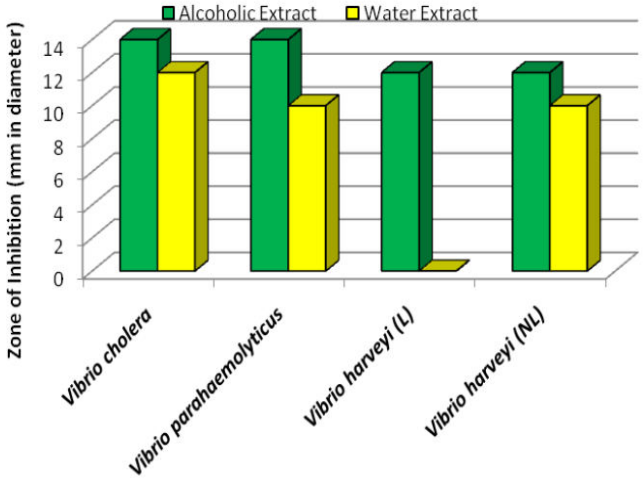

Fig. 1:Inhibition Zone of *Ocimum sanctum* against *Vibrio* species

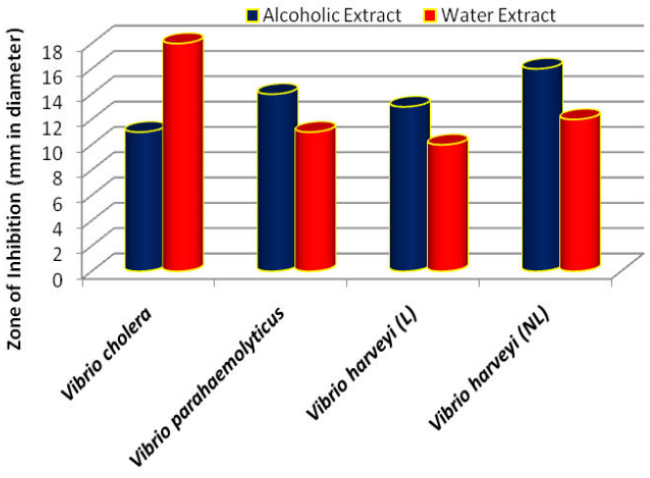

Fig. 2:The Inhibition Zone of *Lucas aspera* against *Vibrio* species

Jaishree *et al.* [17] reported that the aqueous extracts revealed poor bacterial activity. Ethanol extract of *Lucas aspera* was found to be highly active against *Klebsiella pneumonia*, whereas methanol extract was highly active against *Escherichia coli*. In the present study on *invitro* screening of the medicinal plants against shrimp pathogenic Vibrio revealed that among the five plants tested against *Vibrio* spp., the water extract of *Lucas aspera* showed maximum zone of inhibition (18±3.4 mm in diameter) against *Vibrio cholerae*. Among the alcohol extract, the test plants *L. aspera* showed maximum zone (16±3 mm in diameter) of inhibition against *Vibrio harveyi* (NL). Among the five plants tested against *Vibrio* species, the water extract of *Lucas aspera* showed maximum zone of inhibition against *Vibrio cholera* (18±3.4 mm in diameter). Among the alcohol extract, test plants *Lucas aspera* showed maximum zone of inhibition (16±3 mm in diameter) against *Vibrio harveyi* (NL). Hence the present study revealed that the water and alcohol extract of *Lucas aspera* 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.

4. CONCLUSION

The results revealed that the extracts are potent antivibrio against for Vibrio species studied. For all the tested bacteria methanol extract showed maximum antibacterial activity. Hence, the ethanol extract can be used to treat bacterial diseases. The results of the present study provide that, the extract from the leaves of *Lucas aspera* L. using ethanol indicate the antibacterial activity and hence it can be used for the treatment of Vibrio diseases. The crude extract as well as the isolated compounds found to be active in this study could be useful for the development of new antivibrio drugs.

5. ACKNOWLEDGEMENTS

The authors are grateful to PG & Research Department of Zoology, Kundavai Naacchiyaar Govt. Arts College (W) Autonomous, (Affiliated to Bharathidasan University), Thanjavur 613007, Tamilnadu, India offering facilities to carry out this study.

Conflict of interest

None declared

6. REFERENCE

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