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Short Communication

Antibacterial activity of herbal plant extracts towards *Vibrio harveyi*

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Abstract

Fifteen plant extracts were screened against *Vibrio harveyi* using well diffusion method. Methane extract of *Piper betel* showed highest antibacterial activity of 35mm. Due to its less toxicity and potent antibacterial activity, the methanol extract of *Piper betel* may be one of the best options for developing novel antimicrobial compounds for management of diseases caused by *Vibrio harveyi*.

Keywords: *Vibrio harveyi*, plant extracts, well diffusion method

Introduction

Pathogenic *Vibrio* species are naturally occurring bacteria in freshwater and saline environments. Antibiotic therapy is the only prudent method to treat an active *vibrio* outbreak. Unfortunately vibrios are resistant to antibiotics which is a globally alarming problem (Austin, 1993). The frequency of resistance reflects the pattern of antibiotic usage. Moreover natural and synthetic antibiotics produce side effects to the consumers. To overcome this problem, scientists are more interested to develop new antibiotics from unicellular organisms, fungi, algae and higher plants. Among them, higher plants play an important role by producing large number of organic compounds as secondary metabolites, which can be used as self defence. They act as bioactive compounds, chemotherapeutic, bactericidal and bacteriostatic agents (Evans *et al*., 1986). Plant secondary metabolites show activity in the micro to submicromolar range to *Vibrio* (Gibbons, 2005). Plant extracts decrease the selective pressure for developing antibiotic resistance (Lewis and Ausube, 2006). Quinine and berberine from plants remain highly effective to fight against human microbial infections. In the present study plants containing antibacterial activity against *Vibrio harveyi* were studied.

Material and Methods

Plant processing: The plants (15 nos) used in the current study were collected near Vizianagaram, Andhra Pradesh. The leaves were removed and shade dried. After complete dryness they were chopped into small pieces and coarsely powdered in a willy mill (Table 2).

Solvents used: The extraction method employed here is 1 gm of the plant extracts were successively extracted with solvents like chloroform, hexane and methanol basing on their order of polarity using soxlet apparatus. The plant extracts residues were redissolved in 0.1% Dimethyl Sulfoxide to get a concentration of 100 mg/ml.

Test organism: *Vibrio harveyi* was obtained from the Central Institute of Brackishwater Aquaculture (CIBA), Chennai and mainted on Zobell's marine agar (Hi media, Mumbai).

Inoculum preparation: Pure culture of *Vibrio harveyi* was grown on nutrient broth and incubated at 37p C for 24 h.

Assay of antibacterial activity by pour plate technique: Antibacterial activity was carried out by cup plate method (Murray *et al*., 1995) modified by Olurinola (1996). 20 ml of nutrient agar was inoculated into sterile bottles, these were then inoculated with 0.2 ml of culture mixed gently and poured into sterile petri dishes. After setting a number 3 cup borer (6 mm) diameter was properly sterilized by flaming and used to make five uniform cup/wells in each petri dish.

Table 1. The plants and their common names

S.No	Plant	Family	Common Name
1	*Acalypha indica*	Euphorbiciae	Kulci
2	*Adathoda vasica*	Acanthaceae	Vasaka
3	*Albizia lebbecka*	Fabaceae	Dirisana
4	*Carullama* sp.	Apocymaceae	Karallamu
5	*Chinna palleru*	Asteraceae	-
6	*Datura metal*	Solanaceae	Devil's trumpet
7	*Hildergardia populifolia*	Sterculiaceae	-
8	*Lawsonia innermis*	Lythraceae	Henna
9	*Melia azadirach*	Meliaceae	Turaka vepa
10	*Piper betel*	Piperaceae	Betel pepper
11	*Psidium gaujava*	Myrtaceae	Guava
12	*Sidarhombifolia*	Malvaceae	Paddy's lucerene
13	*Siebardia orientalis*	Asteraceae	-
14	*Shilajit* sp.	Lelioceae	Mineral pitch
15	*Terminalia chebula*	Combritaceae	Karakkaya

The cups were filled with 50 µl of the different extracts of 100 mg/ml and allowed to diffuse for 45 minutes. The solvents used for reconstituting the extracts were similarly analyzed. The plates were incubated at 37° C for 24 hrs. Antibiotics like pencillin and streptomycin each 1 mg/ml were also used. The zones of inhibition were measured with antibiotic zone scale in mm.

Results and Discussion

The efficacy of different plant extracts on *Vibrio harveyi* was shown in Table 2. Frequent uses of antibiotics make the organisms resistant to such antibiotics (Sydney *et al*., 1980). Methanolic extracts of *Piper betel* and *Terminalia chebula* exhibited broad spectrum of antibacterial activity. In the present study *Vibrio harveyi* was sensitive to the methanolic extracts of *Piper betel* and *Terminalia chebula* with 35 mm and 22 mm zone of inhibitions respectively (Fig. 1).

The solvents used for reconstitution of the extracts showed no activity. The chloroform extract and hexane extracts were showing less activity when

Table 2. Details of zones of inhibition

Plant	*Vibrio harveyi* (mm)		Antibiotic 1 mg/ml		
	Chloroform	Hexane	Metthanol	Pencillin	Streptomycin
Acalypha indica	-	-	-	-	-
Adathoda vasica	7	8	9	10	-
Albizia lebbecka	-	-	7	-	-
Carullama sp.	-	-	-	-	-
Chinna palleru	-	7	-	10	-
Datura metal	-	-	-	11	
Hildergardia populifolia	-	-	-	9	
Lawsonia innermis	-	9	7	12	
Melia azadirach	-	-	12	-	
Piper betel	-	20	35	-	
Psidium gaujava	7	8	9	15	
Sidarhombifolia	9	9	8	11	
Siebardia orientalis	-	-	-	9	
Shilajit sp.	11	-	-	-	11
Terminalia chebula	16	10	22	-	-

compared with methanolic extracts. Methanolic extracts showed superior activity over *Vibrio harveyi*. Plants have broad spectrum of antibacterial activity against human pathogens (Silva *et al*., 1997 and Navarro and Delgado, 1999). The study supported the claim of the usefulness of the plant extracts in treating diseases in aquaculture caused by pathogenic bacteria like *Vibrio harveyi*.

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