

## Antimicrobial Activity of Medicinal Plants along Kanyakumari Coast, Tamil Nadu, India

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**Abstract:** *In vitro* antibacterial and antifungal activity of the chloroform extracts of the seventeen different coastal medicinal plants were screened against different gram positive and gram negative and fungal ornamental fish pathogens. Of the selected plants *Datura metel* showed wide range of antimicrobial activity against many of the fish pathogens. It is concluded from the present study that, the *Datura metel* which collected from the Kanyakumari coast can be used as a putative antimicrobial drug in the aquaculture maintenance.

**Key word:** Antimicrobial activity • Coastal medicinal plants • Ornamental fish • Microbial pathogens

### INTRODUCTION

A large portion of the world population, especially in developing countries depends on the traditional system of medicine for a variety of disease. Several hundred genera are used medicinally and plants are vital sources for potent and powerful drugs [1]. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, of the phytochemical constituents found *In vitro* to have antimicrobial properties [2]. Many of the spices and herbs used today have been valued for their antimicrobial effects and medicinal powers in addition their flavor and fragrance qualities [3-5].

In India, 500 medicinal plant species are used to pathogenic bacteria [6]. Plants have been used as traditional medicine since time immemorial to control bacterial, viral and fungal diseases. Recently, research has been initiated to evaluate the feasibility of using herbal medicines in fish disease management [7]. Moreover, the bacterial infections are considered the major cause of mortality in aquaculture [8]. Because of the growing bacterial resistance against commercial standard and reserve antibiotics, the search for new active substances with antibacterial activity against pathogenic bacteria is of increasing importance [9, 10]. Recently several reports have carried out with antimicrobial activity against bacteria and fungi [11-15]. Hence the present study was

made an attempt to find out the potential effect of coastal medicinal plants against isolated ornamental fish bacterial and fungal pathogens.

### MATERIALS AND METHODS

**Plant Collection:** Different plant parts (Whole plant, Shoot, Leaf and root) from 17 coastal medicinal plant species belongs to 7 families were collected within the vicinity of 500 M from Mean Sea Level (MSL) along Kanyakumari coast, South West Coast of India (Latitude 8° 4' 60''N and Longitude 79° 34' 0''E). The plants were identified and the voucher specimens were deposited in the herbarium cabinet facility sponsored by Indian Council of Medical Research, New Delhi.

**Extraction:** The collected plant materials were shade dried at room temperature for about 10 days. Then the samples were cut into small pieces and powdered in a mechanical grinder. Extraction (percolation) was carried out with the chloroform solvent for the extraction of bioactive compounds. After 7 days of percolation, the extract was filtered in muslin cloth and evaporated to dry by using rotary flask evaporator (SUPERFIT, INDIA) and the residue obtained was stored in a sterile container kept in refrigerator for further assay. The percentage of extraction from each sample was calculated using the following formula:

Percentage of extraction (%) = Weight of the extract (g) / Weight of the plant material (g) × 100

**Isolation of Ornamental Fish Pathogens:** Infected ornamental fish viz., *Cyprinus carpio* (white carp), *Cyprinus carpio* (orange carp), *Xiphophorus hellerii* (red sword tail), *Xiphophorus maculatus* (red platy), *Poecilia sphenops* (black molly), *Poecilia reticulata* (golden guppy) [29] were collected from small house hold fish farm at friday market, Kanyakumari District, Tamil Nadu, South India. Collected samples were aseptically taken to the laboratory for bacteriological and mycological examination.

The infected part from chosen fishes were cut into small pieces and ground with 10 ml of sterile distilled water with tissue homogenizer. Serial dilutions were made up to  $10^{-8}$ . 1 ml of diluted samples from each dilutions were inoculated on already prepared sterile Rose Bengal agar plates, supplemented with 200 ppm of streptomycin for the isolation of fungi and nutrient agar plates for bacteria. Bacteria inoculated plates were incubated at  $28 \pm 2^\circ\text{C}$  for 24 hrs and fungi inoculated plates were incubated at  $28 \pm 2^\circ\text{C}$  for 3-5 days. After that, the colonies appeared on the media were counted and expressed as Colony Forming Unit (CFU). Then the colonies were restreaked for colony purification and stored at  $4^\circ\text{C}$  for further studies and fungal strains were identified with the help of Smith's introduction to Industrial Mycology identification manual, Seventh Edition [16].

**Antibacterial Assay:** Filter paper disc method was used for testing of medicinal plant extract against 10 ornamental bacterial fish pathogens. Whatman No. 1 filter paper disc (6mm diameter) was impregnated with crude plant extracts ( $5 \text{ mg disc}^{-1}$ ) was placed on Muller Hinton Agar (HIMEDIA, Mumbai) which was previously swabbed with bacterial fish pathogens viz. *Shigella flexneri*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus myxofaciens*, *Vibrio sp.*, *Bacillus cereus*, *Staphylococcus aureus*, *Styphylococcus epidermis*, *Streptococcus pyogenes* and *Clostridium sp.* The sterile disc impregnated with chloroform used as control. All the plates were incubated at  $37^\circ\text{C}$  at 24 hrs under static conditions. After 24 hrs, the zone of inhibition appearing around the discs were measured and recorded in millimeter diameter.

**Antifungal Activity:** The antifungal assay of the extracts was tested against isolated 4 pathogenic fungi viz., *Rhizopus stolonifer*, *Penicillium chrysogenum*, *Penicillium restrictum* and *Aspergillus niger*. The

antifungal activities of the extracts were performed by disc diffusion method with the disc diameter of 6 mm [17]. The molten Potato dextrose agar (PDA) media was then inoculated with 200  $\mu\text{l}$  of the each fungal inoculums ( $10^8$  spores  $\text{ml}^{-1}$ ) and poured into the sterile petri plates. Whatman No. 1 filter paper disc impregnated with crude plant extracts ( $5 \text{ mg disc}^{-1}$ ) were placed on to Potato Dextrose agar (HIMEDIA, Mumbai). Triplicates were maintained for each assay. The plates were incubated for 48 hrs at  $28^\circ\text{C} \pm 2^\circ\text{C}$  and the zone of inhibition was measured. Chloroform alone served as control.

## RESULTS AND DISCUSSION

In the recent years, herbs and herbal products plays significant role in fish culture [20]. The usage of heavy antibiotic in aquaculture field needs to be reduced and replaced with alternative process for treating fish diseases [18]. Alternatively the herbal plants may be used as potential and promising source of pharmaceutical agents against fish pathogens in the organic aquaculture reported by Sridevi *et al.* [19]. Shangliang *et al.* [21] also reported the antimicrobial activity of five Chinese herb extracts against thirteen bacterial and two viral fish pathogens.

The screening results of our study confirm the possible use of medicinal plants as a source of antimicrobial agent for this purpose In the present study seventeen plant parts used and percentage of extract depicted in Table 1. The chloroform extract from 17 plants were tested against identified bacterial and fungal pathogens. It reveals that, 6 out of 17 plants exhibited the promising antimicrobial activity. The coastal medicinal plants showed considerable inhibitory effect ranges from 7- 19 mm against gram positive, gram negative bacterial and fungal pathogens. The highest zone of inhibition was observed in *D. metel* (Solanaceae) against *P. restrictum* fungal species (19 mm) and *E.coli* gram negative bacteria (13mm). Whereas *L. camara* (Verbenaceae) showed antimicrobial activity against six out of fourteen pathogens and *C. inerme* (Solanaceae), *P. guajava* (Myrtaceae) showed antimicrobial activity against five out of fourteen pathogens. *A. calcarata* (Zingiberaceae), *S. cumini* (Myrtaceae) showed antimicrobial sensitivity against two pathogens (Table 2). Shu-tong *et al.* (2001) have screened 88 species of plant extracts against *Phytophthora infestans*, of these, 31 species were completely inhibitory against *Phytophthora infestans*. Moreover 16 kinds of Thai traditional herbs were tested for their antibacterial

Table 1: Showed the Botanical, Local name of the plants and parts used

Botanical name of the plants	Local name	Part (s) used	% of the extract
<i>Cleodendrum inerme</i> (L.) Gaerthn	Changukuppi	Shoot	5.67
<i>Lantana camara</i> Linn.	Poochedi	Shoot	3.89
<i>Amaranthus viridis</i> L.	Kuppakeerai	Shoot	5.24
<i>Amaranthus spinosus</i> L.	Kuppakeerai	Shoot	4.33
<i>Achyranthus aspera</i> L.	Nairuvi	Whole plant	4.56
<i>Aerva lanata</i> Juss ex Schultes	Cherula	Whole plant	3.60
<i>Datura metel</i> Linn.	Ummathu	Leaf	5.02
<i>Blepharis moderaspatisensis</i> L. Roth.	Nethrapoond	Entire	4.20
<i>Solanum indicum</i> L.	Chunda	Leaf	4.58
<i>Asystasia gangetica</i> (L.) T. Anders	Chelanthinagam	Whole plant	4.15
<i>Indoneesiella echioides</i> (L.) Sreem	Gopuramthangi	Whole plant	4.46
<i>Alpinia calcarata</i> (L.) Rose	Chittaratha	Leaf	4.28
<i>Syzygium cumini</i> (L.) Skeels	Naval	Leaf	4.83
<i>Psidium guajava</i> L.	Pera	Leaf	5.11
<i>Syzygium jambos</i> (L.) Alston	Jamba	Leaf	4.49
<i>Spermacoce hispida</i> Linn.	Nathachuri	Whole plant	4.21
<i>Morinda pubescens</i> J.E. Smith	Manjanathi	Leaf	4.61

Table 2: Antimicrobial activities of 17 coastal medicinal plants against ornamental fish bacterial and fungal pathogens

Name of the plant species	Zone of inhibition (mm)													
	Gram Negative Bacteria				Gram Positive Bacteria				Fungal Pathogens					
	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Shigella flexneri</i>	<i>Proteus myxofaciens</i>	<i>Vibrio sp.</i>	<i>Streptococcus pyogenes</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermis</i>	<i>Bacillus cereus</i>	<i>Clostridium sp.</i>	<i>Rhizopus stolonifer</i>	<i>Penicillium chrysogenum</i>	<i>Penicillium restrictum</i>	<i>Aspergillus niger</i>
Verbenaceae														
<i>Cleodendrum inerme</i>	8±0.88	-	7±0.65	-	9±0.90	-	-	-	-	7±0.88	-	6±0.71	-	-
<i>Lantana camara</i>	9±0.90	-	-	7±0.65	10±0.99	7±0.65	-	7±0.65	-	9±1.10	-	-	-	-
Amaranthaceae														
<i>Amaranthus viridis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Amaranthus spinosus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Achyranthus aspera</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Aerva lanata</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Solanaceae														
<i>Datura metel</i>	13±1.02	-	-	10±0.99	10±0.99	-	8±0.77	7±0.65	-	12±1.01	9±0.81	-	19±1.24	7±0.65
<i>Solanum indicum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acanthaceae														
<i>Blepharis moderaspatisensis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Asystasia gangetica</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Indoneesiella echioides</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Zingiberaceae														
<i>Alpinia calcarata</i>	7±0.64	-	-	7±0.55	-	-	-	-	-	-	-	-	-	-
Myrtaceae														
<i>Syzygium cumini</i>	7±0.765	7±0.79	-	-	-	-	-	-	-	-	-	-	-	-
<i>Psidium guajava</i>	8±1.02	-	-	8±0.77	-	7±0.71	-	7±0.65	-	8±0.77	-	-	-	-
<i>Syzygium jambos</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rubiaceae														
<i>Spermacoce hispida</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Morinda pubescens</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Key: - = Absence of inhibition

efficacy against 10 strains of *Vibrio* species, 11 species showed antibacterial sensitivity, out of which 2 of them (*P.guajava* and *M.charantina*) showed the highest effective against *Vibrio* species. In the present study, *Datura metel* showed maximum sensitivity (19 mm dia) against *Pencillium restrictum* fungal species and *Lantana camara* showed maximum sensitivity (13 mm dia) against *Vibrio* species. *Datura metel* Linn (Thorn-apple, Devil trumpet, Solanaceae) is a Nigerian medicinal plant widely used in phytomedicine to cure diseases such as asthma, cough convulsion and insanity [22, 23]. The whole plant particularly the leaves and seeds are used as anesthetic, anodyne, anti-asthmatic, antispasmodic, anti-tussive, bronchodilator and hallucinogenic [22, 23]. It is used in the treatment of catarrh, diarrhea, epilepsy, insanity, hysteria, rheumatic pains, hemorrhoids, painful menstruation, skin ulcers and wounds. These strong antimicrobial activity is might be due to the presence of various phytoconstituents comprise alkaloids, flavonoids, phenols, tannins, saponins and sterols [24-26]. Previously hyoscyamine and scopolamines have been isolated from *D. metel* [27, 28]. It is concluded from the present study that, the chloroform extract of phytoconstituents from *Datura metel* can be effectively used as a potential antimicrobial agents to overcome the problem of mass mortality of ornamental fish in aquarium so as to enable to enhance the market revenue throughout the world.

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