Original Article

Antibacterial effect of *Tradescantia pallida purpurea* against fish (*Labeo rohita*) pathogens

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Abstract:

Diseases are recognized as one of the major restrictions to sustainable animal production which can cause significant economic loss in aquaculture. Even though there are various chemotherapeutic agents such as antibiotics and disinfectants in practice from centuries. Antibiotics have the potential to cause unwanted side effects as well as microbes get resistant against them. Now there is also a lot of research going to see the potential of natural products against pathogens. *Tradescantia pallida purpurea* plant has various bioactive compounds such as alkaloids, flavonoids, tannis and phenolic compounds **Objective:** The present study was conducted to test efficacy of *Tradescantia pallida purpurea* in chloroform extract against fish pathogens and also a comparison in methods (well diffusion and disc diffusion) was made **Methods:** Four fish pathogens (NP1, NP4, SCC4 and TS1) were isolated after morphological and biochemical tests. Erythromycin was used as control. 10 µg/ml chloroform leaf extract was used by well diffusion and disc diffusion **Results:** It was noticed that well-diffusion assay was better to inhibit growth of fish isolates as compared to disc diffusion method. It was noticed that *Tradescantia pallida* has potential to inhibit growth of pathogens. **Conclusions:** So, there is a need to plan a comprehensive study to recommend the maximum dose that is helpful to control fish pathogens.

Keywords:

Aquaculture, antibiotic, potential, natural product

Introduction:

Aquaculture has become an important economic activity in Pakistan. However, spread of fish pathogens results in high mortality levels and nuisance for aquaculture [1]. Antimicrobials and other veterinary drugs are administered as additives in fish diet frequently to elude financial losses [2]. However, the practice of antimicrobial and veterinary drugs is becoming more restricted because of the side-effects for the health safety and environment that could result in the development and growth of resistant strains of bacteria. Human health can be affected by the presence of residual antibiotics in the muscle of commercialized fish [3, 4]. Expensive vaccines are consumed by fish producers for widespread use and they have the disadvantage because a single vaccine can be effective against one type of pathogens [5, 6, 7].

Certain chemotherapeutics are banned in many countries. In these countries the trade of aquaculture products treated with antibiotics and chemicals is also prohibited. Therefore, to improve the health and to enhance the growth performance of cultured fish, the researchers are trying to exploit natural products like plants and herbs in development of alternative dietary supplements instead of chemotherapeutic agents. In fish culture, use of medicinal plants and herbs may be a significant source of therapeutics as these products provides an economical source for treatment without causing toxicity [8-16]. Due to the occurrence of numerous dynamic compounds, plants perform diverse functions. Some of these compounds are phenolics, alkaloids, pigments, flavonoids, steroids, essential oils and terpenoids [17-20]. It

has been reported that plant extracts favor several activities like growth promotion, antistress, appetite stimulation, enhancement of tonicity, anti-parasitic, antimicrobial, aphrodisiac, antioxidant, maturation of culture species, immune stimulating agents as well asanti-pathogen properties in aquaculture of shrimp and fish [21-26].

Tradescantia pallida purpurea has been reported to have seasonal and spatial bio monitoring features for release of metals from vehicle pollution [27]. *T. pallida*, as a household plant has been refereed extremely effective in improving indoor air quality by filtering out volatile organic compounds, the phenomenon is known as phytoremediation. These compounds are common contaminants and cause respiratory disorders. It is also used as an anti-inflammatory and anti-toxic supplement and used for the improvement of blood circulation in Taiwanese traditional medicine [28].Keeping the background knowledge under consideration, present study was aimed to check the efficacy of *T. pallida* leaf extract for its antimicrobial effect against fish pathogens. Moreover, study was used to compare the two different methods such as well diffusion and disc diffusion assay.

Materials and Methods:

Samples collection and extract preparation

Leaves of ***Tradescantia pallida purpurea*** were collected from the botanical garden of Punjab University new campus Lahore. Leave samples were collected, washed with distilled water, air dried and crushed with mortar and pestle and 50 g were added in 200 ml chloroform in a beaker and left for 72 hours [29]. Yield of plant was determined as: Obtained extract/total plant weightX100.

Filtration and Evaporation of Samples

After 72 hours, the whole mixture were filtered twice using muslin filter cloth first and Whatman filter paper (41 pore size) for the removal of fine particle present in the dissolved plant material in order to collect the purified dissolved plant material. The purified plant material extract later be evaporated by placing the beaker in sterilized area stored at 4°C temperature for 24 hours. Four different concentrations were prepared for each of the 0.01 mg of crude extract is dissolved in 1 ml of sterilized distilled water in Eppendorf all this process is done in a sterile area.

Revival of glycerol stock cultures and Evaluated methods

Fish pathogens (TS1, SCC4, NP1, NP4) previously isolated from fish *Labeo rohita* by Qurashi and co-workers were revived from glycerol stock cultures, through swabbing. A fine amount of culture was streaked using inoculating loop and applied at L-agar plates. Plates were kept in incubator at 37ºC for 24 hours and the purity of cultures was checked after 8 h of incubation. Then plates were stored in refrigerator at 4ºC till use. The antibacterial activity of natural products was assessed against four fish pathogens (SCC4, TS1, NP4, NP1). In order to screen the natural products with antimicrobial activity from plant extract (***Tradescantia pallida purpurea***) disc diffusion and well diffusion assay was performed.

Results:

Bacterial isolates already characterized were refreshed by swabbing colonies onto the freshly prepared L- agar plates. Aqueous extract of ***T. pallida*** leaves were obtained with 20 % yield of plant metabolite that obtained using chloroform.

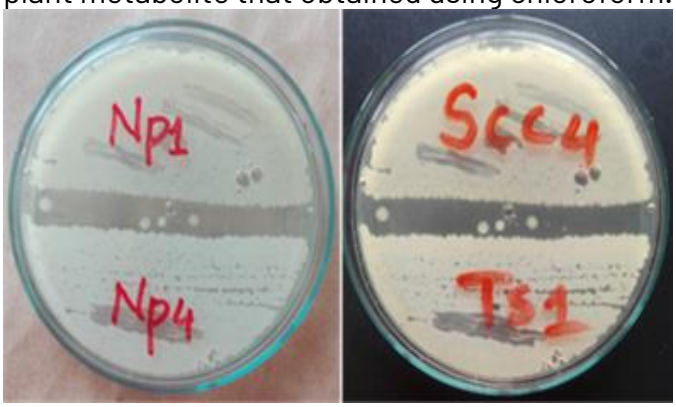

Figure 1: NP1, NP4, TS1 and SCC4 isolated pathogens from fish *Labeorohita*

Antimicrobial activity of the extract of *T. pallida* leaves was tested against fish pathogens using erythromycin as control in both disc and well diffusion assay. Results of disc diffusion assay showed that 10µg/ml extract of *T. pallida* leaves

showed higher growth inhibition (6.5mm) against isolate TS1. Inhibition zone against NP1 is (5mm) which is less than TS1 and higher than NP4(4.5mm) and SCC4(3.5mm). The maximum zone of inhibition recorded with control (Erythromycin) was found higher with all isolated pathogens as compared to *T. pallida.* The zone of inhibition was 12 mm with TS1 and SCC4 while 11mm and 10.5mm with NP1 and NP4 respectively. Results of well diffusion assay showed that 10µg/ml extract of leaves *T. pallida* showed maximum zone of inhibition against isolate TSI (8.5mm) while minimum against NP4. The zone of inhibition against NP1and SCC4 was 6mm which was less than TS1 and higher than NP4. Well diffusion assay with control (Erythromycin) was showed most promising results and maximum zone of inhibition was reported against isolate TS1(17mm) and NP1 (15.5mm).The zone of inhibition against NP4 and SCC4 was recorded same as 15mm. In general antibacterial activity of plant extract was comparatively less as compared to control treatment. Almost similar trend was observed in well diffusion assay as control but this similarity was not recorded in disc diffusion assay. Moreover the zone size was comparatively higher in well diffusion assay as compared to disc diffusion assay.

Growth Inhibition Zones(mm)				
Pathogens(fish)	Disc diffusion assay		Well diffusion assay	
	Extract (10µg/ml)	Control (30µg/ml)	Extract (10µg/ml)	Control (30µg/ml)
NP1	5±1	11±1	6±1	15.5±0.5
NP4	4.5±0.5	10.5±0.5	4.5±0.5	15±0
SCC4	3.5±0.5	12±1	6±1	15±0
TS1	6.5±0.5	12±0	8.5±1.5	17±0

Control:Erythromycin

Table 1: Antibacterial activity of *Tradescantia pallida purpurea*(L) leaf extracts

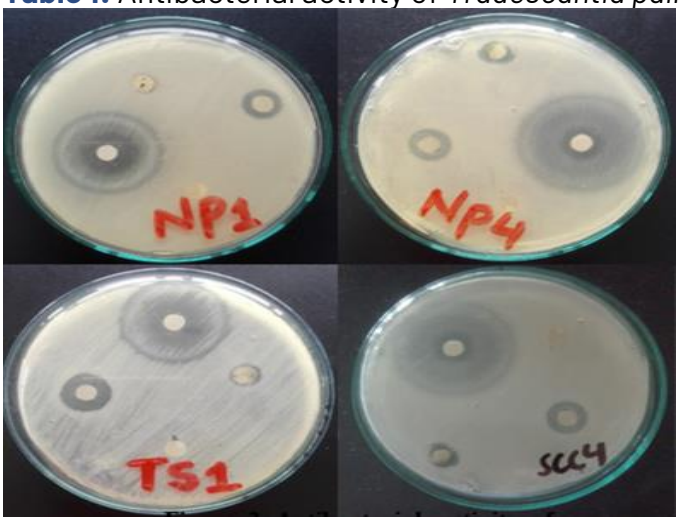

Figure 2: Antibacterial activity of *Tradescantia pallida purpurea* (L) leaf extracts by Disc diffusion Assay

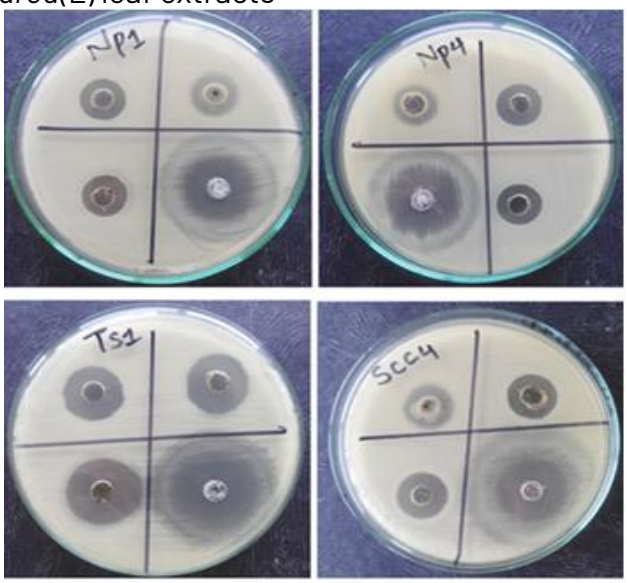

Figure 3: Antibacterial activity of *Tradescantia pallida purpurea* (L) leaf extracts by Well diffusion Assay

Discussion:

Tona *et al*. (1998), reported that plants are significant source of potentially beneficial structures for the growth and development of novel chemotherapeutic agents [30]. According to Rusenova and Parvanov, (2009), the practice of herbal extracts is widely estimated to become an alternate therapy in aquaculture as a prophylactic and to overcome fish diseases [31]. Limited studies are available regarding antimicrobial properties of this herbal extracts against bacteria significantly with fish culture in vitro and in vivo. Hence, the aim of the study was to check antimicrobial efficacy of *T. pallida* leave extract on fish (*Labeo rohita)* pathogens and the result have shown that the aqueous leave extracts of *Tradescantia pallida purpurea (Chloroform)* had antimicrobial activities against the tested fish pathogens (NP1,NP2,SCC4 and TS1). Our present results shows that chloroform leave extract of purple heart plant *(Tradescantia pallida purpurea)* has antimicrobial effects on fish pathogens (NP1,NP2,SCC4 and TS1).

The results of our study showed that plant extract has antimicrobial activity on all of the tested fish pathogens (NP1, NP2, SCC4 and TS1). But both methods (disc diffusion assay and well diffusion assay) give different results for the same pathogen. In our study, we observed the effect of chloroform leaf extract (***Tradescantia pallida purpurea***) against fish pathogens was most promising with well diffusion assay. In previous literature Tan *et al*. (2014) reported that *T. pallida* exhibited the best antibacterial activity against tested fish pathogens, with a MIC of 5mg/mL [32]. Our findings were found parallel to a study conducted by Huq, (2015) that *Tradescantia pallida* plant showed anti-bacterial potential with a zone of inhibition ranging 5-10mm [33].

Natural colorants (such as anthocyanin and annatto) extracted from *T. pallida* have also been recognized in several health applications. It has been based on numerous studies that have described pharmacological, biochemical, and several biological effects such as anti-carcinogenic, antioxidant and antimicrobial activities. The bioactive compounds (alkaloids, flavonoids, tannins and phenolic compounds) present in plant (*T. pallida*). These bioactive compounds exhibit various pharmacological actions like antibacterial, antifungal, antioxidant, anticancer, anti-inflammatory, antiviral, analgesic and pain management. In Taiwanese traditional medicine, *T. pallida* has been used as an anti-inflammatory and anti-toxic supplement and used for the improvement of blood circulation [28].

Conclusions:

Fish aquaculture, (*T. pallida*) plant extracts can be used as a viable and vital substitute for chemical treatments. Moreover well diffusion assay gave better results as compared to disc diffusion assay. The results of our study indicate that supplemented diets with (*T. pallida)* plant extracts of different concentrations have valuable effects on fish health and improved immune system and hence they could play a significant role in aquaculture systems to prevent disease epidemics.

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