International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 7, Issue 6, pp: 63-67, 2022 (Special Issues)
https://doi.org/10.55126/ijzab.2022.v07.i06.sp010
http://www.ijzab.com

Research Article

ANALYSIS ON PHYTOCHEMICAL AND ANTIBACTERIAL PROPERTIES OF HERBAL PLANT EXTRACTS AGAINST FRESHWATER FISH BACTERIAL PATHOGEN (*AEROMONAS HYDROPHILA*)

[1]A. Harish, [1*]R. Raja Jeya Sekar, [1]C. Radhakrishnan Nair, [2]S.M. Vijila, [3]S. Ramesh Kumar

[1]PG and Research Department of Zoology, S.T. Hindu College, Nagercoil-629002.
[2]Pioneer Kumaraswamy College, Nagercoil-629003.
[3]Department of Zoology, Sadakathullah Appa College (Autonomous), Rahmath Nagar, Tirunelveli-627001.
Affiliated to Manomaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.

Article History: Received 08th November 2022; Accepted 12th December 2022; Published 18th December 2022

ABSTRACT

To scrutinize the phytochemical and antibacterial properties of herbal plant extracts of *Psidium guajava, Azadirachta indica, and Terminalia catappa* against fresh water fish bacterial pathogen *Aeromonas hydrophila*. The antibacterial activity was resolved by agar well diffusion method. In this method Isopropyl alcohol extracts of the above three plants exhibited maximum zone of inhibition. The phytochemical properties were evaluated using standard methods. Isopropyl alcohol extract of three plants showed many phytochemical elements. These results showed that the isopropyl alcohol leaf extracts of these plants showed higher antibacterial activity against *A. hydrophila* due to the existence of phytochemical compounds; and hence, it can be used as a substitute remedy against infection caused by *A. hydrophila* in fresh water fishes.

Keywords: Herbal plants, Phytochemical, Antibacterial, *Aeromonas hydrophila*.

INTRODUCTION

Bacterial diseases are a major problem in freshwater fish farming. It causes various types of diseases in fish and also in human beings (Ramesh and Souissi, 2018). *A. hydrophila* is an important freshwater fish pathogen that causes hemorrhagic septicemia and it leads to economic loss in freshwater fish (Vivas *et al.,* 2004). To control these bacterial diseases antibiotics and vaccines are used. The extreme usage of antibiotics leads to the increase of drug-resistant pathogens. It also creates problems for the environment (Austin, 2006). To overcome these problems herbal plant extracts can be used. Many of the herbs have antibacterial activity against pathogenic bacteria (Adithepchaikarn *et al.,* 2008).The herbal plants can be used as a substitute for the simulated chemotherapeutics because it does not affect fish's health and the environment (Mehrim, and Salem 2013; Gabor *et al* 2010).

Phytochemical compounds such as alkaloids, tannins, terpenoids, flavonoids, etc present in herbal plants are responsible to prevent several diseases in fish (Citarasu 2010; Ravikumar 2010; Pandey and Madhuri 2010;Pandey *et al.,* 2012). The antimicrobial activity of medicinal plants having various bioactive compounds provides a vast area for upcoming researchers. The present research prospects the antibacterial and phytochemical properties of three herbal extracts against this infectious bacterium in fishes.

MATERIALS AND METHODS

Collection of fish

Diseased fish *(Catla catla)* was collected from Chittar dam, Alancholai, Kanyakumari district, Tamilnadu.

*Corresponding Author: R. Raja Jeya Sekar, PG and Research Department of Zoology, S.T. Hindu College, Nagercoil-629002 Email: harishajaikumar@gmail.com, Mobile: +91 9944341560.

Isolation of bacteria

Bacterial pathogen *A. hydrophila* was isolated from diseased fish. The collected bacteria were cultured and were sub-cultured in a nutrient agar medium under sterile conditions.

Collection of plant materials

Three herbal plants such as *P. guajava, A. indica, and T. catappa* were collected from villages in Nagercoil, Tamilnadu.

Preparation of herbal plant

The leaves were collected from selected plants and were rigorously washed in distilled water and were allowed to dry under shade. The dried leaf was crushed into a fine powder and stored in airtight containers.

Preparation of herbal plant extract

Five grams of plant leaf powder were taken in a conical flask respectively and were mixed with 50 ml of prescribed solvents (Isopropyl alcohol, Ethyl acetate, Ethanol) was added and kept in an orbital shaker for 24hrs. Then the mixture is filtered through Whatman's No: 1 filter paper. The filtrate was collected in sterile specimen collection containers and then it was kept for evaporation of solvent for 48hrs. After 48hrs the solvents were completely evaporated from mixtures. The remaining powdered extract was weighed and stored at 5ºC for further use.

Identification of *A. hydrophila*

The bacterial pathogen *A. hydrophila* was inoculated in a nutrient agar medium and kept in the incubator for 24 hrs at 37ºC. The bacteria were determined based on the standard procedure given by Bergey and Holt. The different methods for identification of bacteria such as morphological identification, motility, gram staining, indole production, methyl red, citrate utilization, catalase, oxidase, lactose, glucose, trehalose, starch hydrolysis, gelatin hydrolysis, carbohydrate utilization, and urease were analyzed.

Inoculation of *A. hydrophila* with herbal plant extract

According to the standard procedure nutrient, an agar medium was prepared. 9 Petri dish plates were transferred with 20ml of nutrient agar each. The bacteria *A. hydrophila* was incubated with the help of a cotton swab and well-cutting about 1mm diameter was made on a petri dish plate containing nutrient agar. In this well *P. guajava, A. indica, T. catappa* extracts were added individually with the micropipette.

Antibacterial assay

The antibacterial assay was accomplished by the agar well diffusion method.

Phytochemical analysis

Freshly prepared herbal extracts were put through standard phytochemical analysis to find the presence of phytochemical components like alkaloids, tannins, steroids, proteins, etc.

RESULTS AND DISCUSSION

A. hydrophila was isolated from the gastrointestinal tract of freshwater fish and then it was identified by various biochemical tests (Table 1). The morphology of *A. hydrophila* was rod-shaped. Isopropyl extract of *P. guajava* (20mm), *A. indica* (19mm), *and T. catappa* (16mm) showed the maximum zone of inhibition against *A. hydrophila* respectively (Figure 1- 3). Ethanol and Ethyl acetate extract of *P. guajava* (14mm and 12mm), *A. indica* (14mm and 16mm), and *T. catappa* (13mm and 11mm) showed minimum zone of inhibition against *A. hydrophila* respectively (Figure 1-3). The leaf of *P. guajava* exhibited various bioactive components in preliminary phytochemical analysis. In this study, Isopropyl alcohol extract of *P. guajava* leaf exhibited the presence of alkaloids, flavonoids, tannins, carbohydrate, protein, glycoside, and quinones; the ethanol extract of *P. guajava* leaf exhibited the presence of flavonoids, tannins, carbohydrate, and quinones; the ethyl acetate extract of *P. guajava* leaf exhibited the presence of alkaloids, flavonoids, and protein (Table 2).

Table 1. Biochemical analysis of bacteria.

S.No.	Biochemical test	Result
1	Motility test	+
2	Gram staining	-
3	Indole test	+
4	Methyl red test	-
5	Vogesproskaver test	+
6	Citrate utilization test	+
7	Catalase test	+

8	Urease test	-
9	Oxidase test	+
10	Lactose test	+
11	Glucose test	+
12	Starch hydrolysis test	+
13	Gelatin hydrolysis test	+

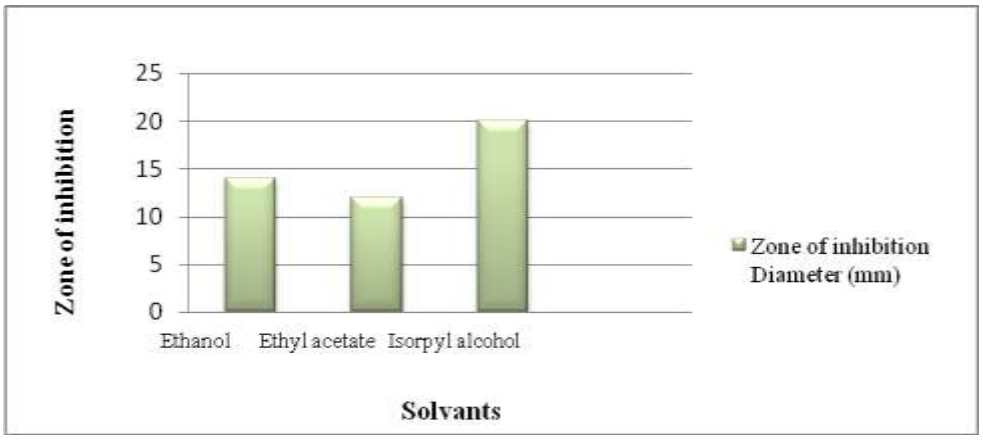

Figure 1. Antibacterial activity of *P. guajava*.

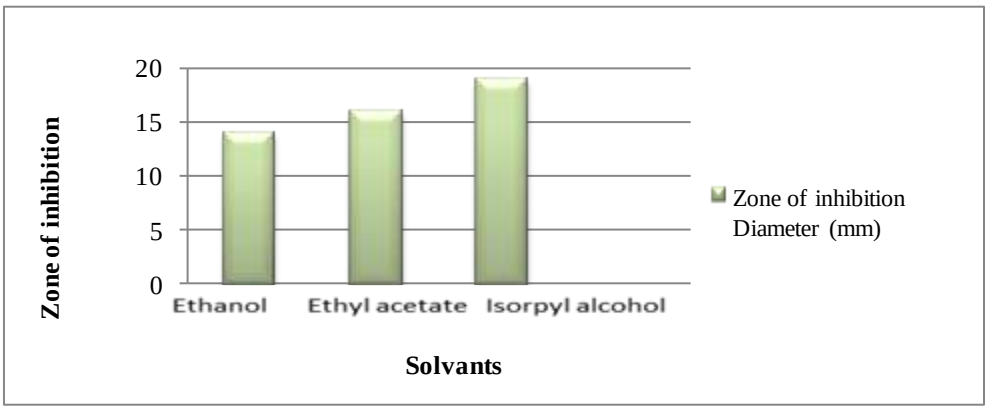

Figure 2. Antibacterial activity of *A. indica***.**

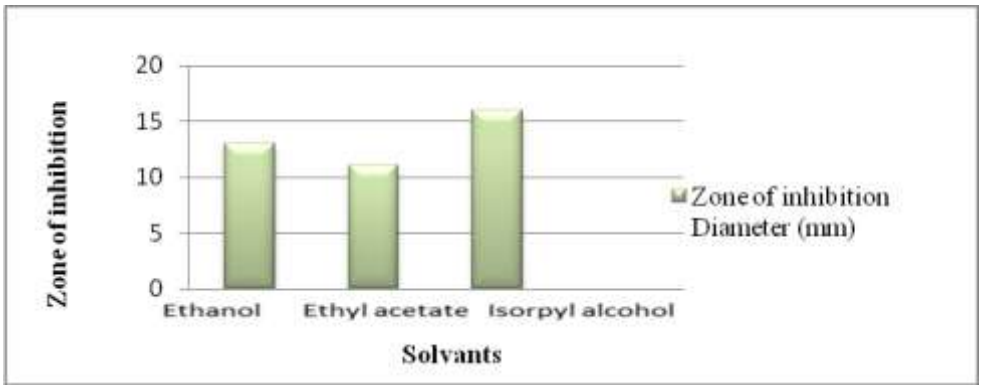

Figure 3. Antibacterial activity of *T. catappa***.**

Table 2. Qualitative analysis of phytochemicals in *P. guajava*

S.No	Phytochemicals	Solvents		
		Ethanol	Ethyl acetate	Isopropyl alcohol
1	Alkaloids	-	+	+
2	Flavonoids	+	+	+
3	Tannins	+	-	+
4	Terpenoids	-	-	-
5	Carbohydrate	+	-	+
6	Protein	-	+	+
7	Carboxylic acid	-	-	-
8	Phenol	-	-	-
9	Glycoside	-	-	+
10	Quinones	+	-	+

(+) present, (-) absent.

The ethanol extract of *A. indica* leaf exhibited the presence of terpenoids and quinones; ethyl acetate extract of *A. indica* showed the presence of flavonoids, tannins, and phenol; isopropyl extract of *A. indica* showed the presence of flavonoids, tannins, terpenoids, phenol, and quinones (Table 3).

Table 3. Qualitative analysis of phytochemicals in *A. indica*.

S. No	Phytochemicals	Solvents		
		Ethanol	Ethyl acetate	Isopropyl alcohol
1	Alkaloids	-	-	-
2	Flavonoids	-	+	+
3	Tannins	-	+	+
4	Terpenoids	+	-	+
5	Carbohydrate	-	-	-
6	Protein	-	-	-
7	Carboxylic acid	-	-	-
8	Phenol	-	+	+
9	Glycoside	-	-	-
10	Quinones	+	-	+

(+) present, (-) absent.

The ethanol extract of *T. catappa* leaf exhibited the presence of terpenoids and phenol; ethyl acetate extract *T. catappa* of showed the presence of alkaloids, flavonoids, terpenoids, and glycoside; isopropyl extract of *T. catappa* showed the presence of Alkaloids, flavonoids, terpenoids, phenol, and glycoside (Table 4).

Table 4. Qualitative analysis of phytochemicals in *T. catappa*.

S. No	Phytochemicals	Solvents		
		Ethanol	Ethyl acetate	Isopropyl alcohol
1	Alkaloids	-	+	+
2	Flavonoids	-	+	+
3	Tannins	-	-	-
4	Terpenoids	+	+	+
5	Carbohydrate	-	-	-
6	Protein	-	-	-
7	Carboxylic acid	-	-	-
8	Phenol	+	-	+
9	Glycoside	-	+	+
10	Quinones	-	-	-

(+) present, (-) absent.

Several diseases which infect freshwater fishes cause an utter loss to the aquaculture (Bondad-Reantaso *et al* 2005). Synthetic antibiotics were used to control fish diseases, but prolonged use caused the development of resistance to pathogens, environmental risk, and bioaccumulation (Rao *et al.*, 2006). The remnant antibiotics in water cause aversion and lethal effects in both habitat and human health (Isnansetyo *et al*., 2009). Medicinal plants serve as the best alternative source for antibiotics in the treatment of diseases in aquaculture (Van Hai, 2015). Herbal plants

were used to develop immune reactions and help to prevent fish diseases and act as a growth-promoting factor in aquaculture (Jeney Galina *et al* ., 2009). The bioactive compounds present in medicinal plants increase the specific and non-specific resistance in fish (Harikrishnan *et al.*, 2012).

CONCLUSION

In this study, the effect of ethanol, ethyl acetate, and isopropyl alcohol extracts of *P. guajava*, A. *indica*, and *T. catappa* leaves was used to restrict gram-negative bacteria *A. hydrophila*. Among these three solvents, isopropyl alcohol extracts of three plants expressed more prevention of *A. hydrophila* and the existence of many phytochemicals constitutes. Relying on these results isopropyl alcohol extracts of all three plants can be used as an influential antibiotic for the control of bacterial diseases of freshwater fishes.

ACKNOWLEDGMENT

The authors express sincere thanks to the Head of the PG and Research Department of Zoology, S.T. Hindu College, Nagercoil for the facilities provided to carry out this research work.

REFERENCES

Randriatsarafara, F. M., Ralamboson, J., Rakotoarivelo, R., Raherinandrasana, A., & Andrianasolo, R. (2015). Consommation d'antibiotiques au Centre Hospitalier Universitaire d'Antananarivo: prévalence et défis stratégiques. *Santé Publique*, *27*(2), 249-255.

Sukenda, W., Widanarni, W., & Setiawati, M. (2016). Immune responses and growth performance of catfish (Clarias gariepinus Burchell 1822) cultivated in bioflok system with different carbon sources and infected with Aeromonas hydrophila. *Jurnal Iktiologi Indonesia*, *16*(3), 309-323.

Austin, B., Austin, D. A., & Munn, C. B. (2007). *Bacterial fish pathogens: disease of farmed and wild fish* (Vol. 26, p. 552). Chichester: Springer.

Pachanawan, A., Phumkhachorn, P., & Rattanachaikunsopon, P. (2008). Potential of Psidium guajava supplemented fish diets in controlling *Aeromonas hydrophila* infection in tilapia (*Oreochromis niloticus*). *Journal of Bioscience and Bioengineering*, *106*(5), 419-424.

Mehrim, A. I., & Salem, M. F. (2013). Medicinal herbs against aflatoxicosis in Nile tilapia (Oreochromis niloticus): Clinical signs, postmortem lesions and liver histopathological changes. *Journal of Aquaculture*, *3*(1), 13-25.

Gabor, E. F., Şara, A., & Barbu, A. (2010). The effects of some phytoadditives on growth, health and meat quality on different species of fish. *Animal Science and Biotechnologies*, *43*(1), 61-65.

Citarasu, T. (2010). Herbal biomedicines: a new opportunity for aquaculture industry. *Aquaculture International*, *18*(3), 403-414.

Ravikumar, S., Selvan, G. P., & Gracelin, A. A. (2010). Antimicrobial activity of medicinal plants along Kanyakumari coast, Tamil Nadu, India. *African Journal of Basic Applied Science*, *2*(5-6), 153-157.

Pandey, G., & Madhuri, S. (2010). Significance of fruits and vegetables in malnutrition cancer. *Plant Archives*, *10*(2), 517-22.

Pandey, G., Madhuri, S., & Mandloi, A. K. (2012). Medicinal plants useful in fish diseases. *Plant Archives*, *12*(1), 1-4.

Bondad-Reantaso, M. G., Subasinghe, R. P., Arthur, J. R., Ogawa, K., Chinabut, S., Adlard, R., & Shariff, M. (2005). Disease and health management in Asian aquaculture. *Veterinary parasitology*, *132*(3-4), 249-272.

Rao, Y. V., Das, B. K., Jyotyrmayee, P., & Chakrabarti, R. (2006). Effect of Achyranthes aspera on the immunity and survival of Labeo rohita infected with Aeromonas hydrophila. *Fish & Shellfish Immunology*, *20*(3), 263-273.

Isnansetyo, A., Istiqomah, I., Sinansari, S., Hernawan, R. K., & Widada, J. (2009). A potential bacterial biocontrol agent, strain S2V2 against pathogenic marine Vibrio in aquaculture. *World Journal of Microbiology and Biotechnology*, *25*(6), 1103-1113.

Van Hai, N. (2015). The use of medicinal plants as immunostimulants in aquaculture: A review. *Aquaculture*, *446*, 88-96.

Galina, J., Yin, G., Ardo, L., & Jeney, Z. (2009). The use of immunostimulating herbs in fish. An overview of research. *Fish Physiology and Biochemistry*, *35*(4), 669-676.

Harikrishnan, R., Kim, D. H., Hong, S. H., Mariappan, P., Balasundaram, C., & Heo, M. S. (2012). Non-specific immune response and disease resistance induced by Siegesbeckia glabrescens against Vibrio parahaemolyticus in *Epinephelus bruneus*. *Fish & Shellfish Immunology*, *33*(2), 359-364.