IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 18, Issue 1 Ser. I (Jan. – Feb. 2023), PP 44-49
www.Iosrjournals.Org

ANALYSIS ON PHYTOCHEMICAL AND ANTI BACTERIAL PROPERTIES OF HERBAL PLANT EXTRACTS (*Murraya koenigii, Azadirachta indica, Phyllanthus emblica)* AGAINST FRESH WATER FISH BACTERIAL PATHOGEN (*AEROMONAS HYDROPHILA*)

Dr .S. Peer Mohamed*[1]
Assistant professor, Department of Zoology, Sadakathullah Appa College (Autonomous), Rahmath Nagar, Tirunelveli-627 011 Affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India

Abstract
To scrutinize the phytochemical and antibacterial properties of herbal plant extracts of Murraya koenigii, Azadirachta indica, and Phyllanthus emblica 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.

Key words: *Herbal plants, Phytochemical, Antibacterial, Aeromonas hydrophila*

Date of Submission: 05-01-2023 Date of Acceptance: 19-01-2023

I. Introduction

Bacterial diseases are a major problem in freshwater fish farming. It causes various types of diseases in fish and also in human beings [1].*A. hydrophila* is an important freshwater fish pathogen that causes hemorrhagic septicemia and it leads to economic loss in freshwater fish [2].

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 [3]. To overcome these problems herbal plant extracts can be used. Many of the herbs have antibacterial activity against pathogenic bacteria [4].

The herbal plants can be used as a substitute for the stimulated chemotherapeutics because it does not affect fish health and the environment [5] [6]. Phytochemical compounds such as alkaloids, tannins, terpenoids, flavonoids, etc present in herbal plants are responsible to prevent several diseases in fish [7] [8] [9] [10].

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 these infectious bacteria in fishes.

II. Materials and methods

2.1 Collection of fish

Diseased fish *(Labeo rohita)* was purchased from Kallidaikuruchi parani private aquarium Tirunelveli district, Tamil Nadu.

2.2 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.

2.3 Collection of plant materials

Three herbal plants such as *Murraya koenigii*, *Azadirachta indica*, and *Phyllanthus emblica* collected from in and around the Tirunelveli district Tamil Nadu, India.

2.3.1 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.

2.3.2 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.

2.4 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.

2.5 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 *Murraya koenigii*, *Azadirachta indica*, and *Phyllanthus emblica* extracts were added individually with the micropipette.

2.6 Antibacterial assay:

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

2.7 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.

III. Results

3.1. Isolation of bacteria

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.

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	Vogesproskauer 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	+

3.2 Antibacterial activity of herbal plant extracts

Isopropyl extract of *Murraya koenigii* (20mm), *A. indica* (19mm), *and Phyllanthus emblica* (16mm) showed the maximum zone of inhibition against *A. hydrophila* respectively. (Fig. 1, 2, 3)

Ethanol and Ethyl acetate extract of *Murraya koenigii* (14mm and 12mm), *A. indica* (14mm and 16mm), and *Phyllanthus emblica* (13mm and 11mm) showed minimum zone of inhibition against *A. hydrophila* respectively. (Fig. 1, 2, 3)

Fig. 1. Antibacterial activity of *Murraya koenigii*

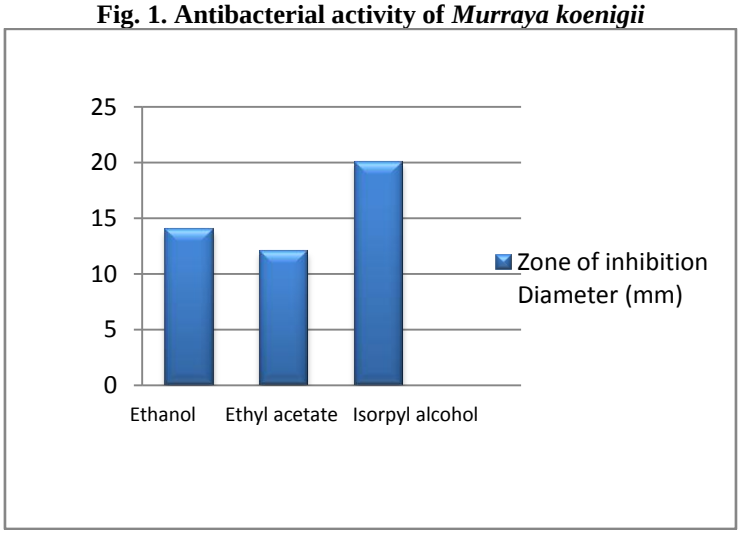


Fig. 2. Antibacterial activity of *A. indica*.

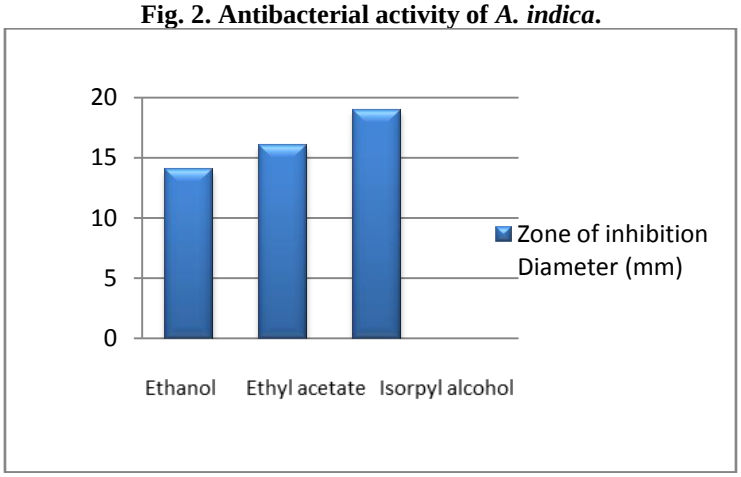
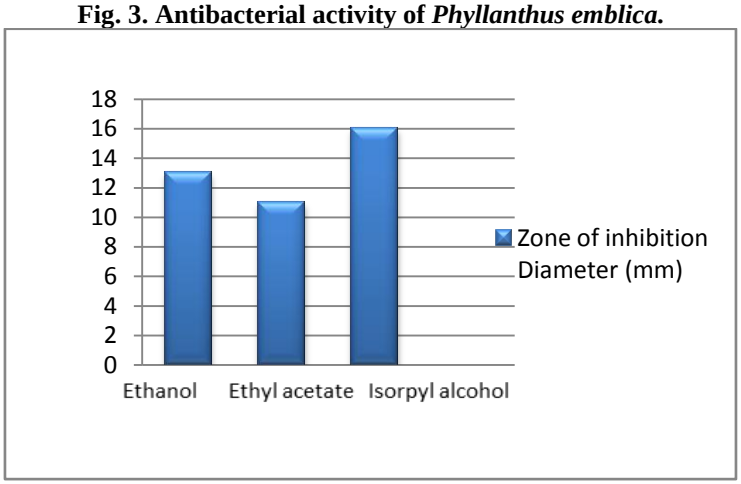


Fig. 3. Antibacterial activity of *Phyllanthus emblica.*



3.3. Phytochemical analysis

The leaf of *Murraya koenigii* exhibited various bioactive components in preliminary phytochemical analysis. In this study, Isopropyl alcohol extract of *Murraya koenigii* leaf exhibited the presence of alkaloids, flavonoids, tannins, carbohydrate, protein, glycoside,and quinines. The ethanol extract of *Murraya koenigii* leaf exhibited the presence of flavonoids, tannins, carbohydrate, and quinines. The ethyl acetate extract of *Murraya koenigii* leaf exhibited the presence of alkaloids, flavonoids, and protein (Table 2).

Table 2 Qualitative analysis of phytochemicals in *Murraya koenigii*

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 quinines. 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 *Phyllanthus emblica* leaf exhibited the presence of terpenoids and phenol. Ethyl acetate extract *Phyllanthus emblica* showed the presence of alkaloids, flavonoids, terpenoids, and glycoside. Isopropyl extract of *Phyllanthus emblica* showed the presence of Alkaloids, flavonoids, terpenoids, phenol, and glycoside (Table 4).

Table 4. Qualitative analysis of phytochemicals in *Phyllanthus emblica*

(+) present, (-) absent.

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	-	-	-

IV. Discussion

Several diseases which infect freshwater fishes caused heavy loss to the aquaculture [11]. Synthetic antibiotics were used to control fish diseases, but prolonged use caused the development of resistance to pathogens, environmental risk, and bioaccumulation [12]. The remnant antibiotics in water cause aversion and lethal effects in both habitat and human health [13].Medicinal plants serve as the best alternative source for antibiotics in the treatment of diseases in aquaculture [14]. Herbal plants were used to develop immune reactions and help to prevent fish diseases and act as a growth-promoting factor in aquaculture [15].

The bioactive compounds present in medicinal plants increase the specific and non-specific resistance in fish [16].

In this study, the effect of ethanol, ethyl acetate, and isopropyl alcohol extracts of *Murraya koenigii*, *Azadirachta indica*, and *Phyllanthus emblica* 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 components. 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.

Reference

[1]. Ramesh D, Souissi S, Antibiotic resistance and virulence traits of bacterial pathogens from infected freshwater fish, Labeorohita, Microbial Pathogenesis (2018), doi: 10.1016/j.micpath.2018.01.019.

[2]. J. Vivas, B. Carracedo, J. rian, et al., Behavior of an Aeromonas hydrophila .A live vaccine in water microorganism, Appl. Environ. Microbiol. 70(2004) 2702-2708.

[3]. Austin B, Austin DA. Bacterial fish pathogens: Diseases of Farmed and Wild Fish; 4thedn. 2006. Springer-Praxis, Chichester, UK.

[4]. A.I. Mehrim, M.F. Salem, Medicinal herbs against aflatoxicosis in Nile tilapia (Orechromis niloticus): clinical signs, postmortem lesions and liver histopathological changes. Egypt. J. Aquae, 3 (2013) 13-15.

[5]. E.F. Gabor, A. Sara, A. Barbu, The effects of some phytoadditives combination on growth, health and meat quality on different species of fish, Scientific paper: Anim. Sci. Biotechnol. 43 (2010) 61-65.

[6]. Citarasu T (2010). Herbal biomedicines: a new opportunity for aquaculture industry. Aquacult Int 18:403-414.

[7]. Ravikumar S, Selvan GP, Gracelin NAA (2010). Antimicrobial activity of medicinal plants along Kanyakumari Coast, Tamil Nadu. Afri J Basic Appl Sci 2:153-157.

[8]. Pandey G, Madhuri S (2010). Significance of fruits and vegetables in malnutrition cancer. Plant Arch 10:517-522.

[9]. Pandey G, Madhuri S, Mandloi AK (2012). Medicinal plants useful in fish diseases. Plant Archives 12:1-4.

[10]. Bondad-Reantaso MG, Subasinghe RP, Arthur JR , Ogawa K, Chinabut S,Adlard R, Tan Z, Shariff M (2005). Disease and health management in Asian aquaculture. Vet Parasitol 132:249-272.

[11]. Rao YV, Das BK, Jyotyrmayee P, Chakrabarti R (2006). Effect of Achyranthes aspera on the immunity and survival of Labeorohita infected with Aeromonas hydrophila. Fish Shellfish Immunol 20: 263-273.

[12]. A. Isnansetyo, I. Istiqomah, I. Muhtadi, S. Sinansari, R.K. Hernawan, R. Triyanto, J. Widada, A Potential bacterial biocontrol agent, strain S2V2 against pathogenic marine Vibrio in aquaculture, World J. Microbiol. Biotechnol. 25 (2009) 1103-1113.

[13]. Van Hai N (2015). The use of medicinal plants as immunostimulants in aquaculture: a review. Aquaculture 446: 88-96.

[14]. Jeney Galina, G. Yin, L. Ardo, Z. Jeney, The use of immune stimulating herbs in fish. An overview of research. Fish Physiol Biochem (2009) 35:669-676.
[15]. Harikrishnan R, Kim DH, Hong SH, Mariappan P, Balasundaram C, Heo MS (2012). Non-specific immune response and disease resistance induced by Siegesbeckia glabrescens against Vibrio parahaemolyticus in Epinephelus bruneus. Fish Shellfish Immunol 33:359-364.

Dr .S. Peer Mohamed. "ANALYSIS ON PHYTOCHEMICAL AND ANTI BACTERIAL PROPERTIES OF HERBAL PLANT EXTRACTS (Murraya koenigii, Azadirachta indica, Phyllanthus emblica) AGAINST FRESH WATER FISH BACTERIAL PATHOGEN (AEROMONAS HYDROPHILA)." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 18(1), (2023): pp. 44-49.