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Comparison of the Antibacterial activities of *Illicium verum* (dried fruit extract) and *Terminalia bellerica* (seeds extract) Against Fish Pathogens

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ABSTRACT

The present study investigated the potential antibacterial activities of the dried fruit extract of *illicium verum* and seed extract of *Terminalia bellerica* in comparison to those of species. Alkaloids, flavonoids, terpenoids, tannins,etc were distributed in two different medicinal families assessed and compared. Minimum inhibition concentration of the various extracts for antibacterial activity was measured against *staphylococcus aures*, *Streptococcus sp, klepsiella sp. E.coli, A.hydrophilla* species. The results of this study indicated the possibility of using the extracts source of antibacterial compounds for treatment of fish infections.

Key words: Antibacterial activity, *Terminalia bellerica*, *Illicium verum*, Fish pathogens, etc...

INTRODUCTION

Fish is one of the cheapest protein among all other important protein food stuff such as eggs, milk, meat and other product constituent excellent source of protein of high biological value, (Cleue, 2008). It was also observed that freshwater fish represent an important source of animal protein to human nutrition. However, the challenge due to pathogenic organisms especially bacteria has limited its effective production and availability. Diseases occurrence in aquatic animal production is beginning to show a significant impact on yield (Hudson, 1990).

Many studies have proved that herbal additives enhanced the growth of fishes and protected them from diseases (Johnson *et al*., 2007). Recently in aquaculture, some plant extracts have been tested and used with good results in the control of bacterial and viral diseases. Therefore, these problems have prompted scientists to search for an alternative to replace antibiotics in controlling diseases in aquaculture.

Herbs are an interesting alternative because they are cheaper than antibiotics. In addition, since herbs may be readily available in local markets or easily grown, user autonomy can increase. Moreover, herbs are often considered environmentally friendly, and therefore, can be used in aquaculture. Research on using herbs to control diseases in aquatic animals is increasing with the demand for environmentally friendly aquaculture processes. Many herbs have promising characteristics for use in controlling fish diseases. Aquaculture is the fastest growing food-producing sector in the world, with an average annual growth rate of 8.9% and practiced in a variety of agro-climatic zones ranging from tropical to temperature area (Subasinghe, 2005).

It includes farming of aquatic organisms, including fish, mollusks, crustaceans and aquatic plants. The world aquaculture has grown tremendously during the last fifty years from a production of less than a million tones to 59.4 million tones to 59.4 million tons (Pannu *et al*., 2013).

The world's total production of fish and shellfish (including mollusks and crustacean) was 99 million tons (mt) in 1990 and it increased to 122 mt in 1990. According organization (FAO) of the United Nations, the global aquaculture production has increased from about 28.3 to 40 mt in 2009 (FAO 2008). Asian countries have witnessed the growth of aquaculture in recent years. The ultimate goal is to produce the greatest possible weight per culture unit in most aquacultural operations for culture fish, crustaceans or mollusc. As aquacultural production becomes more intensive, the incidence of disease including various infectious diseases has increased as a result of it leading to significant economic losses. Diseases are a crucial factor which inhibits the expansion of aquaculture.

Aquaculture, through having a great potential to augment the increasing demand for aqua products, is traversed by the problems in fish disease management. This is evidenced by a host of diseases like motile aeromonad septicemia (MAS)

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and epizootic ulcerative syndrome (EUS) with a cosmopolitan distribution that have sliced down the harvest size in captive and feral fisheries. In aquaculture industry the intensification of fish farming often leads to the emergence of infectious and parasitic diseases that renders disease management a critical factor hampering the development of fish culture in many countries (Thampuran *et al*., 1995).

Among the various diseases, those caused by the pathogenic bacteria represent the gravest threat to aquaculture (Davis *et al*., 1983). Fishes are vulnerable to a wide variety of bacterial diseases like hemorrhagic disease, erythrodermatitis, enteric red mouth disease and epizootic ulcerative syndrome (Jeney *et al.,* 1995).

The incidence of infectious diseases in aquaculture leads to significant economic losses causing significant problems in the development of the sector (Direkbusarakom *et al.,* 1998). Various antimicrobial agents have been used for the treatment of these diseases. However, the use of antimicrobial agents in aquaculture has resulted in the development of more resistant bacterial strains (Cabello *et al*., 2006). In addition continuous use of synthetic antibiotics poses a threat to consumer health, non-target organisms, and the environment (Abutbul *et al*., 2005).

This impressive development has been attended by some practices potentially damaging to human and animal health (Naylor *et al*., 2005). The large-scale settings of aquatic animal husbandry have resulted in an increased antibiotic resistance in bacteria potentially pathogenic to fish and related environment (Cabello, 2006).

The continuous use of antimicrobial agents in aquaculture has resulted in more resistant bacterial strains in the aquatic environment. Continuous use of synthetic antibiotics reveals the threats to consumers and non- target organism in the environment (Abutbul *et al.,* 2005). Treatments of bacterial diseases with various herbs have been safely used widely in organic agriculture, veterinary and human medicine (Direkbusarakom, 2004).

The most common strategy to fight aquaculture disease is the use of antibiotics; however, such usage has been reported to have adverse effects. Drug resistance in fish pathogens can occur and transfer to environmental and human pathogenic bacteria (Abutbul *et al*., 2004). Thus accumulation of antibiotics in fish can be harmful to the environment as well as consumers (Smith *et al*., 1994). Such adverse effects of antibiotics lead to problems in aquaculture; therefore, only few have been approved. Many countries refuse to import cultured products in which antibiotics were used.

Various chemotherapeutants have been used for treatment or prevention of diseases. However, the use of antimicrobial agents in aquaculture has resulted in more resistant bacterial strains. These resistant bacterial strains could have a negative impact on the therapy of fish diseases or human diseases and the environment of the fish farms (Smith *et al*., 1994). Herbs have been widely used in veterinary and human medicine. They are natural products that are not only safe for consumers but also widely available throughout Asia. Nowadays herbs or herbal products also have a significant role in aquaculture.

Oral administration of natural plant products promotes various activities like growth promotion, appetite stimulation, tonic and immune stimulation, and to have antimicrobial properties in some of the aquatic animals due to their bio-active compounds such as phenolics, polyphenols, alkaloids, quinones, terpenoids, lectines and polypeptides and are effective alternatives to antibiotics and other synthetic compounds (Olusola *et al*., 2013).

A large number of medicinal plants have been used for therapeutic and growth promoting purposes in aquaculture (Park, 2012). Indian medicinal plants are a rich source of immune enhancing substances in fish (Galina *et al*., 2009). Aquaculture remains a growing, vibrant and important production sector for higher protein food. The growth of intensive aquaculture production has led to a growing interest in treating or preventing fish diseases. Some plants are rich sources for treatment of cultured fish species and some plants are rich sources of bioactive compounds like volatile oils, saponins, phenolics, tannins, alkaloids, polysaccharides and polypeptides, and these are natural plant products with various activities such as anti-stress, appetizer, anti-microbial, anti-cancer and immune stimulants (Citarasu *et al*., 2006).

Presently, the research has been initiated to evaluate the feasibility of herbal drugs in fish diseases and additionally, the herbal drugs provide a cheaper source for treatment and greater accuracy than chemotherapeutic agents without causing toxicity.

MATERIALS AND METHODS

1. **Collection of plant materials:**

The plants of *Illecium verum* (dried fruit), *Terminalia bellerica* (Seeds) were collected from nearby vegetable shop at Erode. The plants were thoroughly washed in deionized water and were cut into small pieces, dried under shade for 3-4 days and coarsely powdered.

2. Preparation of plant extract:

5g of each fruit plant powder was extracted with 50ml Hot and Cold aqueous and methanol. The extracts were filtered through Whatman filter paper No.1 and it was concentrated using water bath. The yield of each plant extract was calculated. The extract of each plant stored at 4ºC and the extract are used for further study.

3. Qualitative Phytochemical Analysis: (Merina Paul Das *et al.,* 2013).

The extracts were subjected to various phytochemicals tests to determine the active constituents present in the plants.

Detection of alkaloids:

For the detection of alkaloids, aqueous rhizome extract (50mg) was stirred with few ml of dilute hydrochloric acid and filtered. The filtrate was then tested for Mayer's test, Wagner's test and Dragendroff's test (Harborne, 1973).

Detection of Flavonoids:

Sodium hydroxide test and concentrated sulphuric acid test detected the presence of flavonoids (Venkateswarlu *et al.,* 2010).

Detection of Saponins:

Foam test was applied for the detection of saponins in the rhizome sample (Harborne, 1973).

Detection of Tannins:

The phenolic compounds were detected by Ferric chloride test, Gelatin test and Lead acetate test (Harborne, 1973).

Detection of Glycosides:

1ml of conc. Sulphuric acid was taken in a test tube then 5ml of extract and 2 ml of Glacial acetic acid with 1 drop of ferric chloride was added. Reaction shows formation of blue color.

Detection of Steroids:

Two mL of acetic anhydride was added to 0.5g of aqueous rhizome extract with 2ml H_2SO_4. The color changing from violet to blue or green indicated the presence of steroids.

Detection of Terpenoids:

Salkowski test was applied for determination of terpenoids in which formation of a reddish brown coloration of the interface was taken as positive sign for the presence of terpenoids.

Detection of Fixed Oils and Fats:

The presence of fixed oils and fats was detected by Spot test and Saponification test (Harborne, 1973).

Detection of Phytosterols:

The presence of phytosterols was detected by Libermann-Burchard's test and Salkowski reaction (Venkateswarlu *et al.,* 2010).

Detection of Phlobatannins:

An aqueous extract of the rhizome sample was boiled with 1 percent aqueous hydrochloric acid. Deposition of a red precipitate was taken as confirmation for the presence of phlobatannins (Harborne, 1973).

4. Quantitative Estimation of Phytochemicals:

4.1 Estimation of Protein: (Lowry *et al.,* 1951)

100mg of plant dried powder were weighed and macerated in a pestle and mortar with 10 ml of 20 percent trichloroacetic acid. Then homogenate and centrifuged for 15minutes at 6000 rpm. Then the supernatant was discarded. To the pellet, 5ml of 0.2N NaOH was added and centrifuged for 5minutes. The supernatant was served and made up to 10ml of 0.2 N NaOH. This extract was used for protein estimation.Taken 0.3 ml sample from the extraction. Added 0.3ml 2N NaOH, mixed thoroughly, and then kept in a water bath for 10 minutes. After boiling for 10 minutes, cooled at room temperature. Then added 3ml of complex reagent and wait for 10min. Added 0.3ml of folin phenol reagent and the mixture was kept in dark for 30 minutes. The sample was read at 660nm in a UV spectrophotometer. Standard graph was drawn by plotting the concentration BSA on X axis and optical density on Y axis. From the graph the amount of protein presented in the sample was calculated.

4.2 Estimation of Carbohydrates: (Hedge and Hofrieiter, 1962)

Carbohydrates are first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxyl methyl furfural. This compound reacts with anthrone and forms a green colored product with an absorption maximum at 630nm.Weighted 1.0 g of sample taken into boiling tubes and hydrolyzed by keeping

them in boiling water bath for 30minutes to 1 hr with 5ml of 2.5 N HCL. It is cooled to room temperature and neutralized with soiled sodium carbonate until the effervescence ceases and made up the volume to 100ml and centrifuged. Collected the supernatants and 0.5ml and 1.0 ml aliquots were taken for analysis.4ml of 0.2% anthrone reagent is added to each test tube and then it is heated in a boiling water bath for 10minutes.Test tubes are cooled to room temperature; the green to dark green coloration will appear on heating the samples. The optical density (OD) value of the colored solutions is then measured through 630nm wavelength in a colorimeter against blank. Standard graph was drawn by plotting the concentration of glucose on X axis and optical density on Y axis. From the graph the amount of carbohydrate present in the sample was calculated.

4.3 Estimation of Crude Fibre: (A.O.A.C, 1990)

5g of the powdery form of each plants were taken 200ml of 1.25% H_2SO_4were taken 200ml of 1.25% H_2SO_4 were heated for 30 mins and filtered with a Buchner Funnel. The residue was washed with distilled water until it was acid free. 200ml of 1.25% NaOH was used to boil the residue 30 mins, it was Filtered and washed several times with distilled water until it was alkaline free. It was then rinsed once with 10% HCL and twice with ethanol. Finally it was rinsed with petroleum ether three times. The residue was peel in a cruciable and dried at 105ºC in an oven overnight. After cooling in a desiccator, it was ignited in a Muffle Furnance at 550ºC for 90 mins to obtain the weight of the ash.

4.4 Estimation of Ash: (A.O.A.C, 1990)

This was done using the methanol of A.O.A.C (1990). The total ash content of a substance is the percentage of inorganic residue remaining after the organic matter has been ignited. 2g of the Pulverized samples was placed in a crucible and ignited in a muffle furnance at 550ºC for 6 hours. It was then cooled in adesiccator and weighed at room temperature to get weight of the ash.

4.5 Estimation of Flavonoid: (Bohrm and kocipai – Abyazan, 1994)

10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No.42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

5. Isolation of fish pathogens:

The five fish pathogenic bacteria were isolate from the Nile Tilapia fish it was collected from Kulathupalayam, Thindal, Erode. All the bacterial cultures were maintained in nutrient broth and slants. Serial dilutions of all the bacterial cultures were prepared in nutrient medium and used for further studies.

6. Identification of pathogens:

The isolated pathogens were identified on the basis of Gram's reaction and biochemical characteristics and results were identified with the help of Bergey's Manual of systematic Bacteriology.

7. Antibiotic susceptibility test: (Mahendransekar*et al.,* 2013):

The antibacterial activity of the isolated bacterial strains was performed using commercially available antibiotics such as Ampicillin (10µg), Kanomycin (5µg), Ofloxacin (15µg), Erythromycin (5µg), Norfloxin (25µg). A suspension of each strain was swabbed uniformly on each agar plates. Commercially available antibiotics discs were impregnated on the plates and it was incubated at 37ºC for 24 hours. The antibacterial activity was measured by measuring the resulting zone of inhibition against the microorganism.

8. Agar well diffusion technique: (Perez, 1990)

The extract was tested for antibacterial activity by standard agar well-diffusion method against fish pathogenic bacteria *Escherichiacoli, Klebsiella species, Staphylococcus aureus, Streptococcus species, Aeromonas hydrophila*. The pure cultures of bacterial pathogens were sub cultured on nutrient broth; 20ml of nutrient agar were poured into petri plates. Wells of 6mm diameter were made on nutrient agar using gel puncture. Culture was swabbed uniformly using sterile cotton swabs, and then 50µl and 100µl of plant extract solution was loaded into the wells. After incubation at 37ºC for 24 hours, the different levels of zone of inhibition were measured.

9. Minimum inhibition concentration (MIC) – Micro dilution: (Kumarasamy *et al*., 2002)

Minimum inhibition concentration was done by the lowest cost of the extract *Illicium verum, Terminallia bellerica,* where it can show the bactericidal and bacteriostatic effect. The test was performed in 96 well microtiterplate. Microtiter plate wells from each column in row 1 were marked and 100µl (500mg/ml) of stock (aqueous and methanol extract) was added.50µl of steriled

distilled water was added to rows 2-12. Two fold serial dilutions were performed by transferring 50µl of solution from row 1 to 2, using a multichannel pipette. This was repeated down the row 2 to 12. 40µl of double strength nutrient broth and 10µl of bacterial culture was added to all the wells in separate column, so the final concentration of the inoculum in all the wells. To prevent dehydration, the plates were covered with a plastic cover and then incubated at 37ºC for overnight. The bacterial growth was determined after addition of 40µl of 2, 3, 5 Tri Phenyl Tetrazolium Chloride Red (0.02mg/ml). The minimum inhibitory concentration (MIC) of the isolates was taken as the lowest concentration of the antibiotic of which the bacterial tested did not show visible growth.

RESULTS :

YIELDS OF PLANT EXTRACTS

The plants of *Illecium verum* (dried fruit), *Terminalia bellerica* (Seeds), *Acorus calamus* (dried stem) and *Areca catechu* (Seeds), *Murraya koenigi* (Leaves) were taken for the experiment was shown in plate 6.1. Hot water, cold water and Methanol extract of the plants were subjected to the antibacterial activity studies.

The maximal yield was obtained in Hot water extract of *Terminalia bellerica* (1.57g) and the minimal yield was got in cold water extract of *Illecium verum* (0.64g).

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Table 2 and 3 expressed the Qualitative Phytochemical screening for the selected species. Plate 6.2 showed the Alkaloids, Flavonoids and Tannins were present in both the Hot and cold water extract of both plants. Phytosterols, oil and fats and terpenoids were absent in Hot and cold water extract of both plants.

(Nithya devi *et al*., 2014) authenticated the phytochemical analysis of the fruit extracts of *Terminalia bellerica.* It revealed the presence of alkaloids, phenol, tannins and flavonoids in aqueous extract. Tannin, alkaloid, steroid were present in Acetone and ethyl acetate extract of *Illecium verum* reported by (Merina Paul Das *et al.,* 2013).

QUANTITATIVE PHYTOCHEMICAL ANALYSIS

Table 4 was expressed the Quanitative Phytochemical analysis of *Illecium verum* and *Terminalia bellerica*. The crude fiber, ash, Flavonoids, Carbohydrate and Proteins were tested by using plant powders. All the quantities were increasingly present in *Terminalia bellerica* compared to *Illecium verum* except protein content. (Bibhabasu Hazra *et al.,* 2010) reported the flavonoid content of *Terminalia bellerica* was 138.00g.

ANTIBIOTIC SUSCEPTILITY TEST

(Table 5) illustrated the antibiotic sensitivity test against selected pathogens. From the results we concluded that the Erythromycin produced maximum zone against *Klebsiella* (35mm) and the minimum zone was obtained in kanamycin against *Aeromonas hydrophila* (14mm). *Staphylococcus aureus* was highly resistant to all the antibiotics.
In the study of (Thiyagarajan *et al.,* 2014) he reported that the Erythromycin showed maximum zone of inhibition (14-16mm) for *Aeromonas hydrophila*.

MINIMUM INHIBITORY CONCENTRATION

Table 6 to 9 exhibited the Minimum Inhibitory Concentration of cold water extract and hot water extract of selected plants. Both the cold water and hot water extract of *Illecium verum* has higher inhibitory effect against *Aeromonas hydrophila* and *Strepto coccus* (6.25µl).

(Lakshmana swamy parasa *et al.,* 2012) reported the acetone extract produced maximum inhibitory effect against test strains.

Based on the Antibacterial activity and MIC results, among all the plant extracts, *Terminalia bellerica* and *Illecium verum* showed best activity against selected pathogens. So both the plants were choosen for further study.

ANTIBACTERIL ACTIVITY

Antibacterial Activity of *Terminalia bellerica* against selected Fish Pathogens

(Table 7 Figure 2, plate 6.5) explained the Antibacterial activity of *Terminalia bellerica.* The highest zone was observed at 25mm for *Eschrichia coli* and lowest zone was found at 20mm for *Streptococcus* in cold water extract.

The hot water extract of *Terminalia bellerica* have extent sensitivity to *Escherichia coli* (33mm) and least to *Staphylococcus aureus* (21mm). When compared to aqueous extracts, Methanol produced minimum zones against selected pathogens.
Akansha Rana *et al.,* 2014 stated the antibacterial activity of different solvent extracts of *Terminalia bellerica*. He concluded that the ethanol extract

was gave much better zone than the other solvent extracts.

Antibacterial Activity of *Illicium verum* against Selected Fish pathogens

(Table 8) explained the Antibacterial activity of *Illicium verum.* The highest zone was observed at 36mm for *Aeromonas hydrophilla* and lowest zone was found at 18mm for *Strepto coccus* in cold water extarct. The hot water extract of *Illicium verum* have extent sensitivity to *Aeromonas hydrophilla* (38mm) and least to *Staphylococcus aureus* (14mm). When compared to aqueous extracts, Methanol produced minimum zones against selected pathogens.

(Table 10) illustrated the inhibitory effect of *Illecium verum* against selected pathogens. The highest zone of inhibition was observed for cold water extract against *Aeromonas hydrophila* (26mm) and lowest zone obtained against *Streptococcus species* (18mm). In the same way, Hot and methanol extracts produced maximum zones against *Aeromonas bhydrophila* (22mm & 19mm).

Esti *et al.,* 2011 reported the ethanol extract of *Illicium verum* produced 10mm zones against fish pathogens.

(Table 11 Figure 2) explained the Antibacterial activity of *Terminalia bellerica.* The highest zone was observed at 25mm for *Eschrichia coli* and lowest zone was found at 20mm for *Streptococcus* in cold water extract.

The hot water extract of *Terminalia bellerica* have extent sensitivity to *Escherichia coli* (33mm) and least to *Staphylococcus aureus* (21mm). When compared to aqueous extracts, Methanol produced minimum zones against selected pathogens.

Akansha Rana *et al.,* 2014 stated the antibacterial activity of different solvent extracts of *Terminalia bellerica*. He concluded that the ethanol extract was gave much better zone than the other solvent extracts.

Table 1: Yield of Plant Extracts

S.No	Plant name	Yield of Plant Extracts					
		Hot water		Cold water		Methanol	
		g/mg	% of yield	g/mg	% of yield	g/mg	% of yield
1.	*Illecium verum*	0.73g	14.6	0.64g	12.8	1.31g	26.2
2.	*Terminalia bellirica*	1.57g	31.4	1.31g	26.2	0.73g	14.6

Table 2: Qualitative Phytochemical Analysis of *Terminalia bellirica:*

S.No	Parameters	*Terminalia bellirica*	
		Cold water extract	Hot water extract
1.	Alkaloid	+	+
2.	Flavanoid	+	+
3.	Saponin	-	-
4.	Tannin	+	+
5.	Glycosides	+	+
6.	Steroids	+	-
7.	Terpenoids	-	-
8.	Oil and Fat	-	-
9.	Phytostreols	-	-
10.	Phylobatanin	-	+

Table 3: Qualitative Phytochemical Analysis of *Illecium verum:*

S.No	Parameters	*Illecium verum*	
		Hot water extract	Cold water extract
1.	Alkaloid	+	+
2.	Flavanoid	+	+
3.	Saponins	+	+
4.	Tannin	+	+
5.	Glycosides	-	+
6.	Steroids	-	-
7.	Terpenoids	-	-
8.	Oil and Fat	-	-
9.	Phytostreols	-	-
10.	Phylobatannin	-	+

Table 4: Quantitative Phytochemical Analysis:

S.No	Parameters	Medicinal plants	
		Illeciumverum	*Terminalia bellerica*
1.	Flavanoid (g/mg)	0.6	1.7
2.	Crude fibre (g/mg)	0.02	0.03
3.	Ash (g/mg)	0.08	0.09
4.	Carbohydrate(µg/mg)	0.63	0.97
5.	Protein (µg/mg)	0.37	0.25

Table 5: Antibiotic susceptibility test:

S.No	Antibiotics	Zone of Inhibition(mm)				
		Isolates				
		A.hydrophila	*S.aureus*	*S.coccus sp*	*E.coli*	*Klebsiella sp*
1.	Ampicillin(10µg)	R	R	R	R	34mm
2.	Kanamycin(5µg)	14mm	R	15mm	15mm	32mm
3.	Ofloxacin(15µg)	26mm	R	26mm	R	32mm
4.	Erythromycin(5µg)	R	R	R	R	35mm
5.	Norfloxin(25µg)	27mm	R	22mm	R	32mm

'R': Indicates Resistance, 'mm': Indicates milli meter

Table 6: Minimum Inhibitory Concentration of Cold water extract of *Illicium verum*

Sr.No	Isolates	Concentration (500 µg/ml)										
		100µl	50µl	25µl	12.5µl	6.25µl	3.12µl	1.56µl	0.78µl	0.39µl	0.19µl	0.09µl	0.04µl
1	*A.hydrophila*	-	-	-	-	-	+	+	+	+	+	+	+
2	*Klebsiella sp*	-	-	-	+	+	+	+	+	+	+	+	+
3	*S.aureus*	-	-	-	-	+	+	+	+	+	+	+	+
4	*S.coccus sp*	-	-	-	-	-	+	+	+	+	+	+	+
5	*E.coli*	-	-	-	+	+	+	+	+	+	+	+	+

(+) - means presence of bacteria, (-) - Means absence of bacteria

Table 7: Minimum Inhibitory Concentration of Hot water extract of *Illicium verum*

S. NO	Isolates	Concentration (500 µg/ml)										
		100µl	50µl	25µl	12.5µl	6.25µl	3.12µl	1.56µl	0.78µl	0.39µl	0.19µl	0.09µl	0.04µl
1	*A.hydrophila*	-	-	-	+	+	+	+	+	+	+	+	+
2	*Klebsiella sp*	-	-	-	+	+	+	+	+	+	+	+	+
3	*S.aureus*	-	-	-	+	+	+	+	+	+	+	+	+
4	*S.coccus sp*	-	-	-	-	-	+	+	+	+	+	+	+
5	*E.coli*	-	-	-	+	+	+	+	+	+	+	+	+

(+) - means presence of bacteria, (-) - Means absence of bacteria

Table 8: Minimum Inhibitory Concentration of Cold water extract of *Terminallia bellerica*

S. NO	Isolates	Concentration (500 µg/ml)										
		100µl	50µl	25µl	12.5µl	6.25µl	3.12µl	1.56µl	0.78µl	0.39µl	0.19µl	0.09µl	0.04µl
1	*A.hydrophila*	-	-	-	-	-	-	+	+	+	+	+	+
2	*Klebsiella sp*	-	-	-	-	-	+	+	+	+	+	+	+
3	*S.aureus*	-	-	-	+	+	+	+	+	+	+	+	+
4	*S.coccus sp*	-	-	-	+	+	+	+	+	+	+	+	+
5	*E.coli*	-	-	-	+	+	+	+	+	+	+	+	+

(+) - means presence of bacteria, (-) - Means absence of bacteria.

Table 9: Minimum Inhibitory Concentration of Hot water extract of *Terminallia bellerica*

S. No.	Isolates	Concentration (500 µg/ml)											
		100µl	50µl	25µl	12.5µl	6.25µl	3.12µl	1.56µl	0.78µl	0.39µl	0.19µl	0.09µl	0.04µl
1	*A.hydrophila*	-	-	-	-	-	+	+	+	+	+	+	+
2	*Klebsiella sp*	-	-	-	-	-	+	+	+	+	+	+	+
3	*S.aureus*	-	-	-	+	+	+	+	+	+	+	+	+
4	*S.coccus sp*	-	-	-	-	+	+	+	+	+	+	+	+
5	*E.coli*	-	-	-	+	+	+	+	+	+	+	+	+

(+) - means presence of bacteria, (-) - Means absence of bacteria

Table 10: Antibacterial Activity of *Illicium verum* against Selected Fish pathogens

S.No	Isolates	*Illecium verum* (100µl)		
		Cold water	Hot water	Methanol
1.	*E.coli*	24mm	19mm	16mm
2.	*S.coccus sp*	18mm	22mm	21mm
3.	*A.hydrophila*	26mm	38mm	23mm
4.	*S.aureus*	25mm	14mm	24mm
5.	*Klebsiella sp*	20mm	26mm	22mm

'Mm' – millimeter

Table 11: Antibacterial Activity of *Terminalia bellerica* against selected Fish pathogens

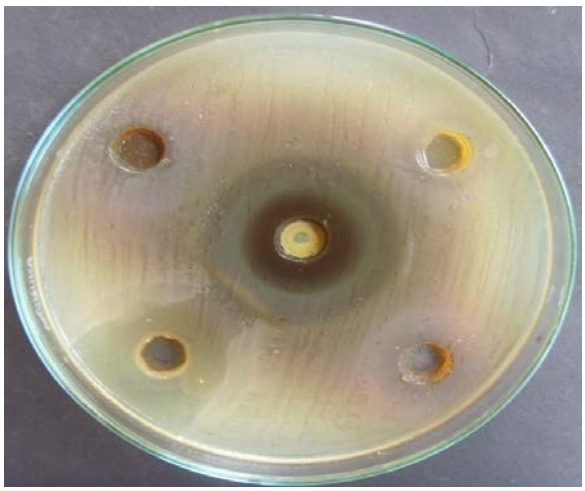
S.No	Isolates	*Terminalia bellirica* (100µl)		
		Cold water	Hot water	Methanol
1.	*E.coli*	25mm	33mm	21mm
2.	*S.coccus sp*	22mm	24mm	20mm
3.	*A.hydrophila*	20mm	21mm	19mm
4.	*S.aureus*	27mm	21mm	18mm
5.	*Klebsiella sp*	22mm	23mm	20mm

'mm' – millimeter

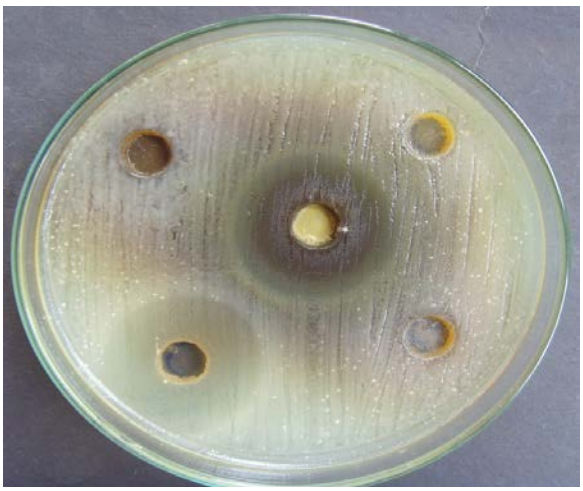
Illecium verum

Terminalia bellirica

Figure 1: dried fruit of *Illicim verum* and seeds of *Terminalia bellerica*



Illecium verum



Terminallia bellerica

Figure2: Antibacterial activity of plant extracts:

CONCLUSION:

In the recent years, the application of herbs to prevent and control microbial diseases has received increasing attention as an alternative treatment of chemotherapeutics. The present study revealed that some herbs have an important role when use mixing with feed to control bacterial diseases in fish. Further detailed studies are necessary before applying herbal therapy by herbal feed in field condition.

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