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Efficacy Of *Ficus benghalensis* Aerial root Against Seafood Pathogens As A Biopreservative

Vijaya Bharathi. S*[1], Rajesh. P[1]

Department of Biotechnology, FSH, SRMIST, Kattankulathur, Tamilnadu – 603203.
Corresponding author: shavisa04@gmail.com

Abstract

Seafood is one of the most important food consumed by more number of population, but the seafood preservation is a big challenge and hence quality control by an effective bio preservative plays a major role in today's safety concept. The current research work focussed to check the bio preservative effect of aqueous extract of *Ficus benghalensis* (aerial root) against the infected fish (*Parupeneus indicus*). Phenolic and flavonoid content of the aerial root extract was determined and also the functional group existence in the extract was confirmed by FTIR analysis. Zone of inhibition of the plant extract against *Vibrio sp* confirmed the effectiveness of *Ficus benghalensis* as a potential antibacterial agent. Microbiological analysis of the preserved infected fish with the aqueous extract of *Ficus benghalensis* at two different temperatures and at different time intervals showed the effectiveness of bio preservative activity.

Keywords: *Ficus benghalensis*, *Parupeneus indicus*, *Vibrio sp*, bio preservative.

Introduction

In Indian Population, seafood consumption is almost more than 60 percent. Among the seafood, fish and prawns are highly consumed by Indian population but the negative side is that the fish marketed is preserved with sodium benzoate, formalin and ice of poor quality which are not safe for health (1). Seafood is a major way that many bacterial diseases are spread. This is because these foods contain pathogenic bacteria that can make people sick. The growing demand for seafood in the national and international also leads to the production of unscrupulous, under-processed, and unhygienic products that may contain bacterial and viral seafood-borne pathogens. When these food-borne pathogens are present, the fisherman and exporters lose a lot of money (2). Seafood pathogens are categorized into two concerns, which are pathogens of major concern and pathogens of minor concern. The major concern agents include; *Vibrio sp, Salmonella sp, Norovirus.* And the minor concern agents include; *Shigella,* Hepatitis A virus (HAV), *Listeria monocytogens, Clostridium botulinum, Staphylococcus aureus* (3). *Vibrio sp,* are natural inhabitants of aquatic environments, infections are usually caused by wounds that have been exposed to seawater or by eating raw seafood (4). Salmonella species cause a lot of illness, death, and disease around the world. Salmonella species infections can lead to a number of different clinical syndromes. All types of salmonellosis in humans are caused by a strong innate immune/inflammatory response from the host (5). Most cases of acute gastroenteritis around the world are caused by noroviruses. Even though they are very infectious, some people in a subpopulation are resistant

to infection and disease (6).Bio preservation is a technique which uses natural or controlled micro biota or antimicrobials by extending the shelf life of the food. In this process, to control and to make pathogens inactive, the beneficial bacteria as well as fermentation products are generally used (7). Acetic acid and lactic acid are the organic acids utilised in bio preservation process(8).

Various elements of the *Ficus benghalensis* plant are broadly used in conventional structures of drugs. Traditionally in ayurvedic medicine the aqueous form of fresh Indian banyan aerial roots are used to enhance the human immune system. This aqueous form is mainly to treat numerous ailments such as diarrhoea, enamel disorders, piles, rheumatism, dysentery, skin and pores problems like hypoglycaemic, to reinforce the immune system and sores. Anti-stress and anti-allergic ability of *F.benghalensis* extracts was studied by Sharma *et al*, 2009 (9).*F. benghalensis* (Indian Banyan tree) has a high-quality medicinal value and has plentiful phytoconstituents like flavanols, flavonoids, terpenoids, sterols, pentacyclic triterpenes and triterpenoids, furocoumarin, tiglic acid ester, coumarins, esters, carbohydrates and serine proteases. Banyan leaf extracts have antidiabetic, hypolipidemic, anthelmintic, antibacterial, immune modulatory and antioxidant residences (10).The purpose of the study is to check the bio preservative efficacy of *Ficus benghalensis*aerial root extract.

Materials and Methods

Preparation of aerial root extract

Ficus benghalensis aerial roots were collected directly from Banyan tree in Pullalur, Kanchipuram district, then it was cleaned, dried and authenicated. The aerial roots were ground into fine powder and stored in an airtight container for further use.Cold Maceration method was followed for extraction of aerial root powder using distilled water as a solvent at room temperature. 100g of *Ficus benghalensis* aerial root powder was mixed with 500 ml of distilled water in a conical flask. The content of the conical flask was mixed thoroughlyat regular intervals for 72 h. The mixture was then filtered using muslin cloth and through Whatman No. 1 filters paper (11). The solvent was evaporated, stored in the refrigerator as a powder sample for further use (FBA).

Characterization of aerial root extract

The powder of *Ficus benghalensis* aerial root extract was further analyzed by FT-IR to analyse the functional groups present between the wavelengths 400 - 4000 cm^{-1}(12).

Estimation of phenolic content

The aerial root extract sample (0.1ml) was added to 0.2ml of 10% Folin Ciocalteau reagent and vortexed by adding the 0.8 ml of sodium carbonate. At room temperature the sample was incubated for 2 h. Absorbance was read at 765 nm (13).

Estimation of flavonoid content

0.5ml of aerial root extract was added with the mixture of ethanol (1.5ml), 10% aluminium chloride (0.1ml), potassium acetate (0.1ml) and distilled water (2.8ml). After incubated for 30 minutes at room temperature then the sample was observed at 420 nm (14).

Fish sample preparation, isolation and characterization of Seafood pathogens

The fish samples (*Parupeneus indicus*) were collected from the local market, Chengalpattu, Tamilnadu. The fish were stored in an ice box and brought to the laboratory within 1 hour. The seafood pathogens were isolated from infected fish samples by homogenizing using sterile distilled water. Then three different dilutions such as 0.1ml, 1ml and 10ml were prepared in test tubes containing sterile alkaline peptone water broth (for 10ml of sample double strength APW was used) for the pre-treatment of Vibrio species. The test tubes were then incubated for 24 hours at room temperature. After the incubation period the cultured samples were

inoculated on sterile TCBS (Thiosulphate citrate bile salt) agar plates. Appearance of yellow coloured colonies indicates the presence of Vibrio species (15).

Biochemical characterization

The respective bacterial isolates stored on MRS agar plates as pure culture and seafood pathogens were subjected to morphological and biochemical characterization by Indole Test, Citrate utilization test and Triple sugar iron test (16).

Antibacterial activity of Ficus benghalensis extract

The Muller Hinton agar plates were prepared and swapped with Vibrio sp then allowed to dry. The wells were cut in agar plates using gel punchers. The samples were loaded in the wells, ampicillin as positive control and methanol as a negative control for *Ficus benghalensis* and Hcl for Zinc oxide and Zinc nanoparticles. The agar plates were incubated for 24 hours at 37℃. After 24 hours of incubation the clear zones were observed and measured (17).

Treatment of fish sample

The fish samples were preserved with aqueous extract of *Ficus benghalensis*. All the treatments of fishes were carried out in polythene bags and maintained at 4℃ and -24℃. Control also maintained without adding the extract(18).

Microbiological load analysis

The part of tissues was cut from all the treated fishes at different time intervals (5th, 10th, and 15th day) and homogenized. Homogenised fish sample was serially diluted up to $10^{-7}$with sterile distilled water. The Total Heterotrophic Bacteria were isolated by using nutrient agar medium and the Vibrio sp were isolated by using TCBS agar respectively (17). All the experiments were carried out as triplicates and standard deviation were calculated. P-value less than 0.05 was considered as statistically significant.

Results and Discussion

Biopreservatives are one of the natural preservative which is to increase shelf life of foods without any side effects. Nowadays, naturally preserved foods have high nutrition and health benefits compared to chemical preservatives because they will have toxicity. In this study, the biopreservation effect of *Ficus benghalensis* was analysed.Quantification of total phenolic and flavonoid content of FBA showed 11.013±1.215 mg/g and 3.351±0.201 respectively.Terpenes, phenolic compounds and alkaloids are the important bioactive compounds found to have preservative property(19).Various review study on natural preservatives of seafoods reports that the plant crude extracts and essential oil of plants plays important role in extending the shelf life of fish (20).It was well known that the flavonoids content plays vital role in inhibiting the microbial growth through different mechanism like inhibiting the synthesis of nucleic acid, cytoplasmic membrane activity, attachment and biofilm formation etc (21). Few flavonoids were identified to reverse the antibiotic resistance and intensify the action of antibiotics (22).

Functional group analysis of aqueous extract of *Ficus benghalensis* aerial root by FT-IR showed the presence of alkyl halides, aliphatic amines, alkanes (Fig: 1). Same type of functional group was also seen in aqueous extract of *Ficus benghalensis* fruit(12).

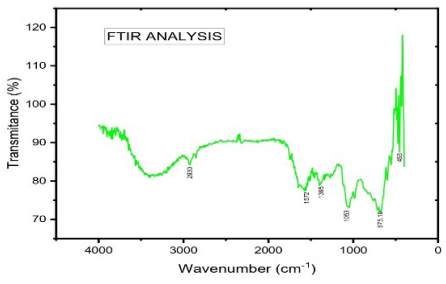

Fig :1 Spectrum of *Ficusbenghalensis*aerial root extract by FT-IR analysis

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The seafood pathogen was isolated using specific media like SS agar and TCBS agar (Fig:2). There was no growth of any colonies in the SS agar plates. Whereas, in TCBS agar plates the colonies were observed in yellow colour which confirms the fish infection with *Vibrio sp*. Further, the biochemical test was done for characterization of *Vibrio sp* which was isolated from the fish sample. The Indole, Citrate Utilization and Triple Sugar Iron test showed positive result which strongly confirmed that the isolated organism was *Vibrio sp*(Fig: 3).

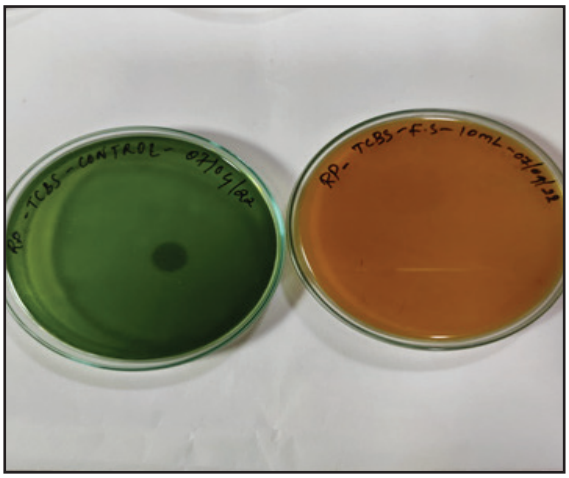


Fig : 2 Isolation of seafood pathogens (Green - control, Yellow - *Vibrio sp.*)

Indole test | Citrate Utilization test | Triple Sugar Iron test

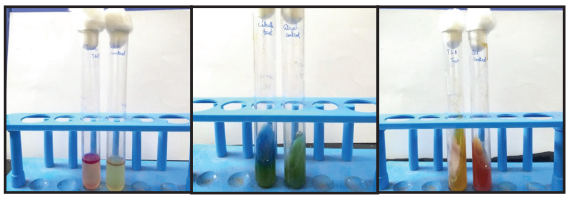
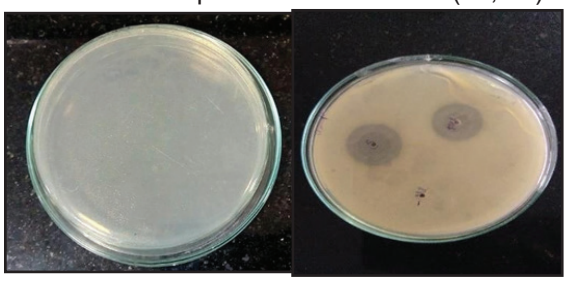


Fig: 3 Biochemical test

The antibacterial activity of the aqueous extract of *Ficus benghalensis*, showed a higher zone of inhibition of 1.5±0.1mm when compared with the positive control (ampicillin) (Fig: 4). Leaves of *Ficus benghalensis* was checked for antibacterial activity against dental pathogens. It was found that the leaf extractwas found effective against *K. pneumonia* than against other pathogens assessed (23).Plant extracts and its bioactive compounds are acquiring importance as biopreservative due to its antimicrobial activity against fish pathogens. In most of the research study against fish pathogen, the ethanol extract was checked and it may be due to the existence of bioactive compounds in the extract (24, 25).



a) b)

Fig: 4 Antibacterial activity of a) Control b) Aqueous of FB

The reduction of bacterial load in the frozen tissues of Indian goat fishwith *Vibrio* infection was observed at 4ºC and -20ºC for 15 days period at 5 days interval. The Total Heterotrophic Bacterial and *Vibrio* counts were enumerated. The observation showed that the THB count was reduced in all the samples preserved with the FBA at both temperatures whereas in the control sample pack no reduction in THB count was seen. Similarly, the bacterial counts of Vibrio were also decreased in the frozen tissues of *Parupeneus indicus* treated with aqueous extract of *Ficus benghalensis* (Fig:5, 6). Overall results confirmed that the infected fish tissues treated with aqueous extract had efficiently reduced the bacterial count which suggested their ability to use as natural bio preservative agent. Plant extracts reported for bio preservative effect were found to be due to the presence of phenolic compounds. Also it was identified that the bio preservative effect of plant extracts were due to its microbial inhibition property and improving texture of the food materials (26).Among the list of Ficus species, *Ficus lyrata* (fruit) and *Ficus carica* (leaves) were checked for its bio presevative effect (27, 28).

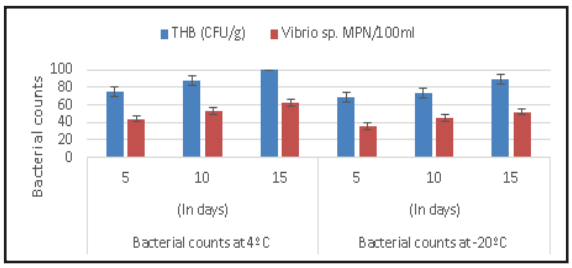


Fig:5 Bacterial counts in *Parupeneus indicus* (Indian goat fish) preserved without treatment at 4ºC & -20ºC

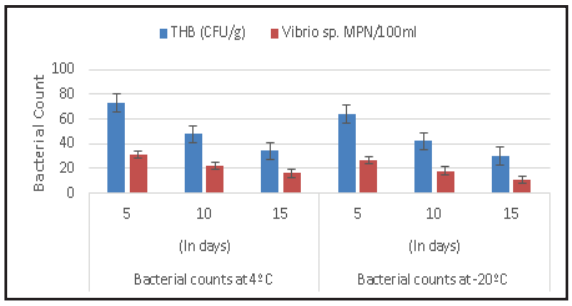


Fig:6 Bacterial counts in *Parupeneusindicus*(Indian goat fish) preserved with *Ficus benghalensis* aqueous extract at 4ºC & -20ºC.

Conclusion

Among the various biopreservatives studied for improving the shelf life of seafoods, plant extracts, essential oil from plants, compounds from animal source, fungus and bacteria are notable. This research work showed the efficacy of Ficus benghalensis as a natural biopreservative and it can form a new revenue in aquaculture.*Ficus benghalensis*aerial root can be an effective biopreservative against seafood pathogen and for the better result, further study understanding the mechanism is needed.

Conflict of Interest

There is no conflict of interest.

Acknowledgement

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