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Antibacterial efficiency of panchagavya against pathogenic bacteria isolated from *Oreochromis mossambicus*

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ABSTRACT

Panchagavya is a combo of five components derived from cow by products. Panchagavya not only enhance the microbes in the environment, it exhibits the synergistic effect. Bacterial pathogens associated with fish can be transmitted to human beings from the fish used as food or by handling the fish. The edible fish of *Oreochromis mossambicus* were processed for the isolation and identification of pathogenic bacteria by following biochemical and Gram staining reactions. Totally 8 bacterial species were identified such as K1 to K8 from fish sample as *Pseudomonas facilis*, *Vibrio parahaemolyticus*, *Pseudomonas vesicularis*, *Edwardsiella tarda*, *E. coli*, *Aeromonas hydrophilla*, *Streptococcus aureus* and *Vibrio* sp. The objective of this study was to evaluate the antibacterial activity of panchagavya against the pathogenic bacteria isolated from *Oreochromis mossambicus*.

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INTRODUCTION

Panchagavya is a term used to describefive major substances obtained from cow, which includes cow's urine, milk, ghee, curd and dung. Panchgavya therapy or cowpathy utilizes these five products, as these possess medicinal properties and are used singly or in combination with some other drugs of herbal, animal or mineral origin for treatment of several disorders and diseases (Abulreesh *et al.,* 2004). According to the ancient Indian medical system known as Ayurveda, cow's milk, curd, ghee and urine are all specifically noted as being crucial for treating a range of human illnesses. Every product has distinct qualities and uses in the fields of agriculture, human health and animals (Anami *et al.,* 2012; Baby and Sankarganesh, 2011). The nutrient-rich composition of Panchagavya promotes plant growth, enhances fertility of the soil and improves the microbial population in the soil, ultimately leading to sustainable agriculture (Kuo *et al.,* 2003). A panchagavya Ayurvedic formulation containing *E. officinalis, G. glabra* and cow's ghee was evaluated for its effects on mice's spontaneous motor activity, rota-rod performance (motor coordination), pentobarbital-induced sleep duration, pentylenetetrazol-induced seizures and amphetamine antagonism (Cheesbrough, 1984). When combined with a Newcastle disease vaccination program, Panchagavya reduced the clinical signs and severity of Newcastle disease in layer chickens (Deepika *et al.,* 2016).

Panchagavya-mediated Cu-NPs, demonstrating their potential for antimicrobial, antioxidant, anticancer, and larvicidal applications, which could contribute to sustainable pest and disease management strategies (Eze *et al.,* 2011). Cow urine acts as an integral component of Panchagavya in enhancing immune responses as have been tested by various workers (Kajal *et al.,* 2020; Karthiga *et al.,* 2016). Cow urine, or distillation of it, has been shown to have many positive health effects, including increased longevity and improved quality of life for those suffering from life-threatening illnesses (Kumar *et al.,* 2004). The use of different kinds of livestock manure in fish production may increase the level of pathogenic bacteria causing a public health risk to the rural community (Mathivanan *et al.,* 2006). The transmission of these pathogens to people can be through improperly cooked food or the handling of the fish. There have been great economic losses reported due to food borne illness such as dysentery and diarrhea resulting from consumption of contaminated fish and such can be a problem to the immune compromised, children and elderly people (Mathivanan and Kalaiarasi, 2007).

The microbial association with fish compromises safety and the quality for human consumption; particularly critical is when the micro-organisms are opportunistic and/or pathogenic in nature (Natarajan, 2003). There may be a potential risk of infection from food borne diseases to the residents from the surrounding communities from consuming the fish from the earth dams. Fish should be viewed not only as food, but also as a ready source of income in the smallholder farming sector (Obi and Krakowiaka, 1983). Integrated fish farming combines livestock production with fish farming. Animal manure is shed directly into a fish pond as fertilizer and supports the growth of photosynthetic organisms (Jaya *et al.,* 2008). While supplemental feeding affects fish growth directly, fertilization contributes to growth via the planktonic natural food. In addition to acting as a food for fish, plankton perform other important functions in pond aquaculture: a net producer of dissolved oxygen, which is indispensable for fish growth and the most important sink of ammonia-nitrogen, which is excreted by fish (Harathi *et al.,* 2017). Panchgavya products have been found to be beneficial in curing several human ailments and enhance the body's immunity and resistance to fight various infections (Petronillah *et al.,* 2014).

These circumstances prompted this research to isolate and identify the pathogenic bacteria associated with freshly caughted edible fish *Oreochromis mossambicus*. In the current study, antibacterial activity was performed to find out the efficiency of

panchagavya against the pathogenic bacteria isolated from fish.

MATERIALS AND METHODS

Study area

For this study, the fish samples were collected from Lower Anaicut, Annaikarai. It is situated 70 miles below the upper Anaicut and 25Km from Kumbakonam. About 40 fish samples were collected under aseptic condition in sterile polythene bags in the early morning. The fishes were processed within 3 hrs of acquisition, followed by storing in refrigerator at 4 to 8˚C.

Preparation of sample from fish (*Oreochromis mossambicus)*

Sample preparation was made using the method described previously (Prashith *et al.,* 2010). The bacterial counts on the external surfaces, intestines and tissue were estimated. 10g of the fish sample from skin, intestine, gills and tissues was dissected out, blended and mixed properly in a pestle and mortar. It was aseptically transferred to a sample bottle containing 9ml of 0.1 % sterile peptone water. From the crushed sample, 1 ml aliquot volume was measured out and homogenized in a clean, dry sterile beaker containing 9 ml of distilled water giving a 1:10 dilution.

Isolation and identification of bacteria from fish

After incubation, the colonies underwent biochemical, microscopic, macroscopic and continuous streaking purification tests to determine their properties. Using the previously mentioned morphological and biochemical traits, the organisms were identified (Parkavi *et al.,* 2021). In addition to gram's staining behavior, shape and cell organization. The bacterial isolates were analyzed under macro and microscopy on nutrient agar plates for colony morphology, surface pigment, size, margin and surface. The isolated bacterial colonies were identified on the basis of their morphological, physiological and biochemical characters. These culture were subjected to various biochemical tests such as gram staining, indole, methyl red, voges proskauer, citrate, Triple sugar ion, oxidase, carbohydrate fermentation, hydrogen sulfide production tests for identification of phosphate solubilizing bacteria using Bergey's manual of systematic bacteriology (Silliker and Gabis, 1976).

Preparation of panchagavya

In accordance with the methods described in (Slaby *et al.,* 1981), the panchagavya was prepared using cow dung (5%), urine (3%), milk (2%), curd (2%) and ghee (1%) in addition to additional ingredients such sugarcane juice (three parts), tender coconut juice (three parts), ripened bananas (12 Nos). For three days, the fresh dung was completely combined with ghee in a wide-mouth mud pot. Once every day, the aforesaid combination was well stirred. Other ingredients were added to the mud pot on the fourth day, thoroughly mixed and covered with nylon net to keep flies out. Twice daily for 28 days, the pot was kept in the shade and properly mixed.

Antibacterial activity

In this experiment, the isolates of infected fish samples were used for determining the antibacterial activity (Sumithra *et al.,* 2013). In Muller-Hinton agar plates, the appropriate bacteria were grown for 24 hours using sterile cotton swabs. Using a sterile cork borer, agar wells (5 mm in diameter) were created in each of these plates. To minimize the effects of variations in time between the applications of different solutions, standard concentrations of panchagavya was added using sterilized dropping pipettes into the wells. The plates were then left for an hour to allow for pre-incubation diffusion. The plates were then incubated for 24 hours at 37˚C ± 2˚C for bacteria. The presence of an inhibition zone was recorded and calculated.

Statistical analysis

The experiments were done three times. The results are shown as average values with their standard deviations.

RESULTS AND DISCUSSION

In the present study, the fish sample were serially diluted, spread out on Mueller and Hinton agar plates, diluted factors is 10^{-4}, 10^{-5}, 10^{-6} and incubated at 30˚C for 3 days. The maximum numbers of colonies were observed under the dilution factor of 10^{-4} and 10^{-5} showed 147 colonies (CFU/ml), 10^{-6} showed (TLTC) Too Low Too Count. According to (9), this organism (*E. coli*) is especially helpful as a contamination indicator when it occurs in small numbers or as a mishandling indicator when it appears in large numbers. The outcome reveals that the dilution factor with clear colonies visible in each plate ranged from 10^{-5} to 10^{-2} dilution for the twenty crushed frozen fish samples represented by S1 to S20. The mean number of colonies per dilution factor, on the other hand, ranged from 1 to 32 colonies. The total plate count (TPC) for all the fish samples ranged between 3.60 × 10^4 and 23.60 × 10^4cfu/g. Out of the 150 fish samples analysed for TPC, the skin had the highest number of bacteria with 23.60 × 10^4 cfu/g at Nyamakwe. The gills had the lowest isolation with 3.60 x 10^4 cfu/ml at the Imbayago sites. The Coliform count was highest in Nhengo (19.66 × 10^4 cfu/g) as compared to other location. The isolation of *Pseudomonas sp*. with the skin having the highest number in *Oreochomis mossambicus* (26.60 × 10^3cfu/g) at (Vinay *et al.,* 2019).

In the current study, we propose designating to the bacteria that were isolated from fish sample as strain K. The strains were selected to perform pure culture to grow into eight clearly defined colonies (K1 to K8). K2 and K6 were rapid growth and remaining slow growth was noted. K2 and K5 were punctiform shape and balances for circular shape were recorded.K3, K4, K6, K7 and K8 colonies showed smooth shiny colonies and the rest of the colonies were rough in surface. In order to identify the bacterial strains, a variety of biochemical tests were performed. Totally 8 bacterial species were identified such as K1 to K8 from fish sample as *Pseudomonas facilis, Vibrio parahaemolyticus, Pseudomonas vesicularis, Edwardsiella tarda, E. coli, Aeromonas hydrophilla, Streptococcus* aureus *and Vibrio* sp.

The intestines were notably some of the most colonized parts in the fish with having the highest count of 23.30 x 10^3 cfu/g. The gills likewise showed possible colonization but in the lowest count as compared to other parts. No isolation of *Vibrio* spp. on the gills of the fish. The intestine and gills were also heavily populated by *E. coli* with the highest exhibited in the gills of fish isolated from Nhengo (6.4 × 10^3 cfu/g). Likewise, the intestines exhibited the highest *Streptococcal* colonization rate of 17.64 × 10^3 cfu/g. *Vibrio* spp. had the lowest counts which are largely insignificant (Yagoub, 2009).

In the present study, the isolated bacteria were identified by Gram staining technique were found to be Gram positive and Gram negative. The results revealed that Gram positive *Streptococcus sp*. and Gram negative organisms were *Pseudomonas facilis, Vibrio parahaemolyticus, P. vesicularis, Edwardsiella tarda, E. coli, Aeromonas hydrophilla* and *Vibrio* sp. was the most abundant pathogenic bacteria associated with the fish in *Oreochomis mossambicus*. The presence of *Staphylococcus aureus* was attributed to the contamination of the fish samples by man through handling and processing as suggested (Saxena *et al.,* 2004).

The presence of coliforms in fish demonstrates the level of pollution of their environment because coliforms are not the normal bacterial flora in fish. Of the organisms that were isolated and identified that is *S. typhi, S. aureus, S. dysenteriae* and *E. coli* are non-indigenous pathogens that contaminate fish or fish habitats in one way or the other (Achliya *et al.,* 2004; Chauhan, 2005). The most common pathogenic bacteria found in this environment associated with frozen fish were *S. aureus, E. coli,* and *L. plantarum*, in that order. The infection of the fish samples by people with *S. aureus* was attributed. (Samuthirapandi *et al.,* 2025) observed that *S. aureus* seldom, if ever,

occurs as natural microflora of fish and shellfish; rather, its primary home is people and other animals, where it is mostly found in the skin, nose and throat of healthy individuals.

Nyamakwe fish ponds had the highest number of bacteria compared to other ponds. The reason might be suggested that they are using more cattle manure (90kg/ha/week) compared to other livestock wastes (Holt *et al.,* 1994). The isolation of *Salmonella, Shigella* and *E. coli* indicate faecal and environmental pollution (Janak *et al.,* 2024). Coliforms such as *E. coli* are usually present where there has been faecal contamination from warm blooded animals (Clucas and Ward, 1996). The organism *E. coli* is recognized as the reliable indicator of faecal contamination in small numbers and in large numbers it is an indicator of mishandling (Eze *et al.,* 2011).

Panchagavya solutions are useful in healing numerous human disorders and enhancing the immune system's ability and resilience to battle against infections. The panchagavya demands the attention of scientific circles for its verified validation, ubiquitous recognition, propagation, and popularity (Sumithra *et al.,* 2013). According to the investigations of (Deepika *et al.,* 2016), panchagavya evaluates its antibacterial activity against an extensive spectrum of microbial infections. In Indian Ayurveda, remedies involving panchagavya are pretty well-known. Its various qualities and medicinal benefits from curd, milk, cow dung, ghee and urine are abundantly mentioned in ancient Ayurvedic writing (Cheesbrough, 1984).

Antibacterial activity of panchagavya for the bacterial isolates obtained from fish sample has been determined by agar well diffusion method. The maximum zone of inhibition against *Aeromonas hydrophia, Edwarsiella tarda, E. coli, Pseudomonas facilis, Vibrio* sp, *Pseudomonas vesicularis, Vibrio parahaemolytics* and *Streptococcus aureus* which is shown in the Table 1. In the treatment of diseases due to pathogenic bacteria (*Bacillus subtilis*, *S. aureus*, *Escherichia coli* and *Enterobacter aerogenes*); opportunistic fungi (*Aspergillus niger*) and helminthes (intestinal roundworm), CUC is found to be highly effective (Harathi *et al.,* 2017; Prashith *et al.,* 2010).

Table 1. Antibacterial activity of different concentration of panchagavya against isolated bacteria from fish

Name of the bacteria	Zone of inhibition (mm)			
	Different concentration (µl)			
	100	200	300	400
Pseudomonas facilis	11.10±0.05	11.35±0.23	12.40±0.12	12.80±0.50
Vibrio parahaemolyticus	10.65±0.11	10.70±0.56	10.93±0.84	10.55±0.32
Pseudomonas vesicularis	10.25±0.19	10.35±0.08	10.50±0.41	10.96±0.13
Edwardsiella tarda	14.10±0.06	14.61±0.34	15.20±0.74	15.30±0.17
Escherichia coli	10.85±0.04	10.90±0.23	11.35±0.51	11.95±0.11
Streptococcus aureus	10.60±0.21	10.65±0.03	10.20±0.52	10.11±0.12
Aeromonas hydrophila	15.10±0.16	15.20±0.14	15.30±0.45	15.40±0.13
Vibrio sp	10.65±0.12	10.90±0.47	10.95±0.06	11.10±0.05

The values were expressed in terms of (Mean ± Standard deviation)

The curd and buttermilk are enriched with a lot amount of lactic acid bacteria. The presence of lactic acid bacteria also produces antimicrobial metabolites (Anami *et al.,* 2012). There is a considerable zone of inhibition for cow dung was observed only in *Staphylococcus aureus*. Cow urine from different cows had a different level of antimicrobial properties. The difference in the level of antimicrobial properties of different cow urine may be because of difference in chemical composition of urine which may arise due to several reasons (Vinay *et al.,* 2019).

CONCLUSION

Panchagavya is an organic formulation from cow products and probably the greatest strength of traditional agriculture. Panchagavya was tested for its

antimicrobial activity against various pathogenic bacteria isolated from *Oreochromis mossambicus* fish samples. Panchagavya enhances the microflora level in water bodies; meanwhile it induces the antibacterial activity against the certain bacteria which cause diseases in fish. Panchagavya ensures minimal soil and water pollution. So it helps in the conservation of environment.

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