ANTIBACTERIAL EFFECT OF LEMON PEEL *(Citrus limon)* EXTRACT ON SPOILAGE CAUSING BACTERIA IN TILAPIA (*Oreochromis niloticus)* FISH

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Abstract: Spoilage is defined as any alteration in food products that lead to unacceptable attributes to the consumers, mainly perceived through sensory evaluation. After the fish dies, thousands of microscopic organisms that are invisible to the naked eye are present on the layer of its slimy skin and in its intestines. These microorganisms start to multiply and are known as spoilage bacteria. The aim of the study was to identify antibacterial activity of lemon peel extract against bacteria isolated from gills, skin and intestine of fresh Tilapia fish. Thirty (30) samples were collected in clean polythene bags from old market, Wukari Taraba State and the samples were taken to the laboratory for bacteriological analysis. The bacterial loads of the samples were determined using the Pour plate method of inoculation. The colonies obtained were sub-cultured repeatedly to obtain pure cultures. The isolates were identified using gram staining technique and biochemical tests. The organisms isolated were *Staphylococcus aureus*, *Pseudomonas aerunginosa*, *Bacillus subtilis* and *Escherichia coli*. The total colony counts from the intestine, gills and skin were too numerous to count, 4.40×10^4 and 3.25×10^4 respectively. *Escherichia coli* (41.37%) had the highest occurrence while *Bacillus subtilis* (17.24%) had the least. lemon peel extract was applied against the isolated bacteria using the disc diffusion method under in vitro conditions. The disc diffusion results indicated that extract of lemon peel significantly inhibited the growth of *Staphylococcus aureus*, *Pseudomonas aerunginosa*, *Bacillus subtilis* and *Escherichia coli*. Our results suggested that the use of lemon peel extract induced a stronger antibacterial effect.

Keywords: Spoilage, Bacteria, Tilapia fish, Lemon peel, Antibacterial, Preservative

Introduction

Spoilage is defined as any alteration in food products that lead to unacceptable attributes to the consumers, mainly perceived through sensory evaluation (Rawat, 2015). The vulnerability of aquatic food products to microbial spoilage is due to the presence of high moisture content and nutrient availability (Singh *et al.,* 2021). The spoilage of fish begins with the release of its body fluids. At the initial stage of spoilage, the development of a sickly-sweet odor can be observed, followed by stale-fish odor caused by trimethylamine, then by ammonia odor, and finally, putrid odors owing to hydrogen sulfide and indole (Erkmen & Bozoglu, 2016). Microbial spoilage is responsible for the 30% losses of landed fish worldwide (Kuley *et al.,* 2017). It has been reported by Ghaly *et al*. (2010) that fish spoilage is caused by three basic mechanisms, including enzyme autolysis, oxidation, and microbial growth. The spoilage of fresh fish can occur very rapidly after it is caught and as fish spoils, it breaks down into various components and forms new compounds. The new compounds cause fish flesh to change smell, taste, and different texture (Ghaly *et al*., 2010). After the fish dies, thousands of microscopic organisms that are invisible to the naked eye are present on the layer of its slimy skin and in its intestines (Diwan *et al.,* 2022). Normally the surface of fish skin has a total count of 10^2 -10^7 (CFU)/cm2 of bacteria, whereas its intestine and the gills both contain 10^3 -10^9 CFU/g of bacteria, and when it dies, these bacteria start to multiply (Rathod *et al.,* 2022). These microorganisms are known as spoilage bacteria. Fish from temperate and tropical oceans as well as those from fresh and marine waters may have a range of spoilage bacteria, which are the most prevalent agents of spoilage and are mostly responsible for the deterioration of aquatic food products (Laorenza *et al.,* 2022; Prasai *et al.*, 2022). If these bacteria are not eradicated, they will continue to destroy the fish until it is completely spoiled, making them the adversary of everyone concerned in the production of fresh fish (Mir *et al.,* 2022). One of the factors that promote the growth of spoilage bacteria in fish is the temperature which is regarded as the most important factor that encourages bacterial multiplication (Abraha *et al.,* 2018). Bacteria multiply as the temperature rises, and it only takes a few hours under the sun's warmth to start showing signs of deterioration (Ali *et al.,* 2022). In fish, bacterial proliferation results in a slimy coating of skin, discoloration of the eyes and, most noticeably, the gills, which is visible throughout the entire fish, and, finally, a loss of muscle texture as a result of proteolysis, which causes the muscles to softened (Torell *et al.,* 2020). In regard to the foul and spoiled smell of fish, bacteria aid in the putrefaction of the proteins, caused by the unpleasant odor (Abdel-Aziz *et al.,* 2016).

There are various methods of retarding or delaying spoilage in aquatic food products. These include the use of traditional techniques of processing aquatic food products, such as drying, brining, fermentation, salting, and smoking (Arason *et al.,* 2014; Tahiluddin & Kadak, 2022), and non-traditional methods, such as chilling, freezing, and canning (Jessen *et al.,* 2014). More recently, the application of microwave energy processing has been highlighted to process aquatic foods (Viji *et al.,* 2022). Moreover, the potential application of natural preservatives in the prevention of spoilage, thereby extending the shelf-life of aquatic food products, has been reported and well-documented (Mei *et al.,* 2019). Plants have been traditionally used to treat various ailments and infections and plant extracts form a pool of potential sources of antimicrobial compounds containing diverse secondary metabolites or phytochemicals, including phenolic compounds, terpenoids, and alkaloids,

which contribute to their antimicrobial properties (Radulovic *et al.*, 2013). Citrus fruits are an excellent source of antioxidants including phenolic compounds, ascorbic acid, essential oils, and carotenoids and thus can potentially prove helpful to prevent cellular oxidative damage (Vlaicu *et al.*, 2020). The peels of citrus fruits make an almost 50–60% of the total weight of citrus fruits. The percentage of refined citrus fruits out of the total production is just 33%, whereas thousands of tons of citrus fruit peels ultimately become as agro-industrial waste (Singh *et al.*, 2020). Several previously reported studies also show that in most of the citrus fruits, the compounds responsible for antioxidant properties are mainly present in their peels rather than in their pulps (Czech *et al.*, 2021). To avoid food spoilage, synthetic preservatives are most commonly employed but the use of these synthetic preservatives is harmful for human beings as well as for the environment (Singh *et al.*, 2021; Nasrollahzadeh *et al.*, 2022). Citrus peel extract, according to recent studies, has a stronger inhibitory effect on lipid oxidation and a better antioxidant activity than synthetic antioxidants. Synthetic antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertiary butylhydroquinone (TBHQ) are also unstable at high temperatures (Singh *et al.*, 2020). Lemon peel is a cost-effective, easy-to-find waste material known as GRAS (generally recognized as safe) and is associated with antimicrobial characteristics (Yazgan *et al.*, 2019). lemon (*Citrus limon*) leaves extracts have been demonstrated to exhibit antibacterial properties against human pathogenic bacteria with the extracts containing phenolic compounds that may have contributed to their antibacterial potentials (Ewansiha *et al.*, 2016, Asker *et al.*, 2020). A study conducted on the inhibitory effects of citrus lemon oil against *Streptococcus sobrinus* found evidence of some level of inhibition ability on the pathogen (Liu *et al.*, 2020).

Fish is widely produced, marketed, and consumed around the world due to its favorable taste, efficient feed conversion, high commercial value and nutritional properties as it is a source mainly of protein and polyunsaturated lipids (Ali *et al*., 2022). It is estimated that fish constitutes approximately 17% of the animal protein intake of the world population (Dos Santos Silva and e Barros, 2020), providing important nutrients for physical and cognitive development, from fetal growth to early childhood, childhood and maintaining good nutrition and health during adolescence and adulthood (FAO, 2022). The Nile tilapia *(Oreochromis niloticus*) has grown phenomenally in popularity and has become the second most abundantly farmed fish, behind carps (FAO, 2018). The culture of this species is produced in large quantities worldwide (Abdel-Tawwab *et al.*, 2020), due to its modest requirement for artificial feeds (Ogello *et al*., 2014) and absence of intermuscular bone (Moesch *et al.*, 2016). Even though tilapia fish is highly nutritive, it acts as a vehicle for the pathogenic bacteria occurring naturally in the marine environment, bacteria are also responsible for a short shelf life of the tilapia fish (Gillespie *et al*., 2011). Because of its high demand and circulation, it is necessary to consider ways to increase its shelf life by reducing the spoilage causing bacteria. Hence, the need to determine the efficacy of Lemon peel extracts as antibacterial agents against spoilage causing bacteria in Tilapia fish.

Materials and Methods

Study Area

The study was carried out in Wukari Local Government Area of Taraba State North East, Nigeria. It lies between latitude 7^{O} 51′ N to 7^{O} 85′ Nand longitude 9^{O} 46' E to 9^{0}78 E' of the Greenwich meridian. The entire area is a gently undulating plain, with a mean altitude of 200 m above sea level. The drainage systems drain northward and serve as tributaries network to the river Benue while eastward discharge of rivulets and other smaller tributaries from Wukari town drains towards the Donga River, which is a major tributary of River Benue. The mean annual rainfall value ranges from 1000 - 1500 mm. The onset of the raining season is usually around April while the offset period is October. The mean maximum temperature is being experienced around April at about 40^{O} C while the mean minimum temperature occurs between the period of December and February at about 20^{O} C (Osujieke *et al.*,2018).

Sample Collection

Thirty (30) samples of fresh Tilapia (*Oreochromis niloticus*) fish were collected in clean polythene bag from old market Wukari, Taraba State. The samples were transported to the Department of Biological Sciences Laboratory, Federal University Wukari and separated into two groups; the treatment group and the control group. Both the treatment and control groups were labeled appropriately and stored in refrigerator at 4 °C for further analysis as described by Van Rensburg *et al.* (2013). For antibacterial activity, fresh lemons bought from the new market Wukari were taken to Biological Sciences Laboratory Federal University Wukari.

Preparation of Media

Nutrient agar (for total bacteria count) and MacConkey agar (for total coliform count) were used and prepared according to the manufacturers' instructions. The exact quantity of the media was weighed and dissolved in the appropriate volume of sterile distilled water. The suspension was heated to boil and was sterilized using an autoclave at 121ºC for 15 minutes (Odo *et al.*, 2023).

Determination and Isolation of Bacterial Loads

The bacterial loads of the samples were determined using the Pour plate method of inoculation (Odo *et al.*, 2023). A sterile knife was used to dissect the fish under aseptic condition and the samples (gills and intestine) were serially diluted where 9ml of sterile distilled water was measured into 5 test tubes labeled 10^{-1} to 10^{-5} and was properly mixed to have a homogenized mixture (stock solution). 1ml was taken from the stock sample and was transferred into the first tube labeled 10^{-1}, this was mixed properly and 1ml was taken out of the same tube (10^{-1}) and was transferred into the tube labeled 10^{-2}. This was done serially down to the last tube labeled 10^{-5} and 1ml was discarded from the last tube. exactly 0.5ml of the fish part samples using a sterile pipette was introduced into the surface of already prepared nutrient agar (for total bacteria count) and MacConkey (for total coliform count). For isolation on skin, a sterile cotton swap was used and streaked onto the surface of already prepared agar plate. The plates were labelled as A for bacterial isolates from Skin, B for bacterial isolates from Gills and C for bacterial isolates from the Intestine. The plates were aseptically left undisturbed for some minutes for the medium

to solidify and were inverted and incubated at 37°C for 24 hrs. The colonies obtained after 24 hours of incubation, were counted using a colony counter and recorded in colony forming unit per milliliter (CFU/ml). The broth cultures were sub-cultured onto MacConkay agar medium and were incubated for 24 hrs and the colonies obtained were further subcultured repeatedly to obtain pure cultures. The growth characteristics of the isolates on each growth medium were observed and recorded as described by Mohammad *et al.* (2021) and Odo *et al.* (2023).

Gram Staining Technique

Colony morphology and Gram staining were carried out according to the methods described by Valencia *et al*. (2019) and Odo *et al.* (2023). The colony of each isolate was emulsified using a wire loop, in a drop of normal saline on a clean microscope slide (smear). It was air-dried and was heat-fixed by passing the slide across a flame three times. The slide was placed on the staining rack was flooded with crystal violet for 1 minute and was rinsed with clean water. It was then covered with lugol's iodine (mordant) for 1 minute and rinsed with clean water. It was rapidly decolorized with acetone and was rinsed immediately with clean water. It was lastly stained with safranin (secondary dye) for 1 minute. The back of the slide was wiped with tissue and was allowed to air dry; the slide was mounted on the microscope stage and a drop of immersion oil was added. The specimen was brought into focus using a 100x objective with the iris diaphragm completely opened to increase the amount of light that entered the objective. Gram reaction was determined on the basis of the color of the dye retained; Gram-positive organisms retained the color of the primary dye (crystal violet) and appeared purple while Gram-negative organisms lost the color of the primary dye after decolorization and picked up the color of the secondary dye (safranin) and appear red in color.

Biochemical Tests

The isolates were further identified using various biochemical tests/characteristics as described by Cheesbrough, (2009) and Mondal *et al*. (2010). Pure cultures of the isolates were identified by biochemical characterization using the criteria outlined in Bergey's Manual of Determinative Bacteriology (Vos *et al.*, 2011).

Catalase test

A drop of hydrogen peroxide was placed on a microscope slide and the isolate was picked using a wire loop and was emulsified in the drop of the hydrogen peroxide and was observed for immediate bubble formation with indicated catalase positive result.

Coagulase test

The slide method as described by Cheesbrough, (2009) was used to carry out the test. A drop of distilled water was placed on a slide and colonies of the test organism were emulsified to make a thick suspension, and a loopful of plasma was added to the suspension and mixed gently and was observed for agglutination within 10 seconds. Agglutination within 10 seconds indicates coagulase coagulase-positive result.

Indole test

The test organism was inoculated in a test tube containing 5ml of sterile peptone water and incubated at 37°C for 48 hours 0.5ml of Kovac's reagent was added and shaken gently and was examined for red color formation on the surface layer within 10mins. The formations of red color on the surface layer indicated an indole-positive result (Cheesbrough, 2009: Vos *et al.*, 2011).

Methyl red (MV) test

The isolated colonies were inoculated into glucose phosphate broth, which contains glucose and a phosphate buffer and incubated at 37^0C for 24 hours. The pH of the medium was tested by the addition of 5 drops of methyl red reagent. Red or yellow colour change indicates a positive reaction, while green or blue indicates a negative reaction (Vos *et al*., 2011)

Citrate utilization test

The media was prepared in a test tube and placed in a slant position. The butt was stabbed using a sterile straight inoculation needle and the slope was streaked with a saline suspension of the organism and incubated at 37°C for 24hrs. A change in color from green to bright blue is indicative of a citrate-positive result (Vos *et al*., 2011).

Preparation of Lemon Peel Extract

The procedure for preparation of extracts was carried out as described by Atikya *et al*. (2014). The lemon was washed well using tap water followed by sterile distilled water. The peel was separated and cut into small pieces then it was air dried for a period of 6 days at room temperature. To obtain the powdered form, the dried sample was ground using mortar and pestle. 300g of dry plant material was soaked in 150mls of distilled boiled water (hot water extract) for 24hrs. The slurry obtained was left in clean glass container and shaken vigorously to allow for proper extraction. The slurry was filtered using a sterile muslin cloth after which the extract obtained was air dried and stored at 4^0C until required.

Assessment of Antibacterial Activity of Lemon Peel Extract

The antimicrobial activity of the extract was evaluated *in vitro* by disk diffusion assay as described by Saravanan and Parimelazhagan (2014). Nutrient agar was prepared according to the manufacturer's instruction. The isolated bacteria colonies were placed on the agar plates by spread plate method using a sterile inoculating needle. Different dilutions of the lemon peel extract were prepared in the order of 0.1, 0.2, 0.3, 0.4 and 0.5g/ml respectively in test tubes and placed on a test tube rack. Disks that were soaked with the different dilutions of the extract were inoculated unto the medium and allowed to stay for up to an hour for proper diffusion of plant extract before incubating it at 37^0C for 24hours. Tetracycline was used as the positive control and water used as the negative control. The resulting inhibition zones were measured in millimeters and recorded against the corresponding concentrations.

Statistical analysis

Percentage Occurrence of the bacterial isolates was calculated. Results for antibacterial activity of lemon peel extract were expressed in terms of mean ± standard error of three replicates and analyzed with one-way analysis of

variance (ANOVA) followed by Duncan's multiple range test, a post-hoc test. Statistical analysis was done using SPSS version 23. P-values of less than 0.05 ($P<0.05$) were considered significant.

Results and Discussion

Table 1 shows the total bacteria counts. The highly infected part of the examined *Oreochromis niloticus* was the intestine with a colony which was too numerous to count as compared to the skin and the gills with colony counts of 3.25×10^4 Cfu/ml and 4.40×10^4 Cfu/ml respectively. The colony counts suggest that the bacteria load in the Tilapia fish is highly significant and could pose a great risk to the consumers who usually eat them because the infective dose of these bacteria can be dangerous to humans when consumed, often leading to food poisoning (Muhammad *et al.,* 2020). This corroborates with the findings of Odo *et al.* (2023) and Muhammad *et al.* (2020). Ajani *et al.* (2016) and Albert *et al*. (2016), also reported high bacterial loads in the intestine part of African Catfish. The bacterial load present in gills and on skin may be due to contamination as a result of indiscriminate deposition of waste materials into the river through runoffs, animal and human excreta and other environmental wastes. The bacterial load on the skin may be due to the fact that the skin is always in contact with the surrounding water and the skin may get contaminated with bacteria during handling. This observation agrees with the work of Osuntokun *et al. (*2020).

Table 1: Total Bacteria Counts

Fish organ	Bacterial population (Cfu/ml)
Intestine	TNTC
Gills ($\times 10^4$)	4.40
Skin ($\times 10^4$)	3.25

Note: TNTC= Too numerous to count

The bacterial isolates were identified by microscopic examination and biochemical tests. The identified bacterial isolates were *Staphylococcus aureus, Pseudomonas aerunginosa, Bacillus subtilis* and *Escherichia coli* (Table 2 and 3). The occurrence of these bacterial species in different organs of fish could be an indication of presence of certain predisposing factors such as contamination during fish harvesting and handling. Live healthy fish can be colonized by bacteria while the flesh remains sterile. After death, incorrect or inadequate handling can introduce bacteria to the flesh resulting in spoilage. Similarly, poor handling may lead to contaminating fresh fish with man natural flora such as *Staphylococcus aureus* and *Eshcherichia coli* (Osuntokun *et al.,*2020; Muhammed *et al.*, 2020). Foodborne bacteria such as *Escherichia*, *Bacillus*, and others grow mostly at room temperature and are mostly dominated in fish (Coimbra *et al.*, 2022). Additionally, *Pseudomonas* species are among the major spoilage bacteria at near freezing temperatures (Adebayo *et al.*, 2012; Rathod *et al.*, 2022) while Bacillus spores can survive and reproduce especially in unsalted fish (Chitrakar *et al.*, 2019). The high number of *Eshcherichia coli* 12(41.37%) is an indication of fecal contamination which could be attributed to the poor sanitary condition and unhygienic practices by the vendors in the study area. This study is similar to a study carried out by Odo *et al*. (2023), who reported the presence of pathogenic organisms like *Salmonella typhi* and *Eshcherichia coli*, suggesting organisms that are transmitted via the fecal-oral route.

Table 2: Identified Bacterial Isolates of *Oreochromis niloticus*

Bacteria	Total number of isolates	Percentage (%)
Staphylococcus aureus	6	20.68
Bacillus subtilis	5	17.24
Escherichia coli	12	41.37
Pseudomonas aerunginosa	6	20.68

Table 3: Characterization of bacterial isolates of Tilapia fish (*O.niloticus*)

Test	Bacteria Isolates			
	S. aureus	***E. coli***	***P. aerunginosa***	***B. subtilis***
Gram staining	Positive cocci	Negative, bacilli	Negative bacilli	Positive bacilli
Catalase	Positive	Positive	Positive	Positive
Coagulase	Positive	Negative	Negative	Negative
Indole	Negative	Positive	Negative	Negative
Methyl red	Positive	Positive	Negative	Negative
Citrate	Positive	Negative	Positive	Positive

Antibacterial activity was determined by measuring the diameter of the inhibition zone. The larger the diameter of the inhibition zone, the better its antibacterial activity (Retnaningsih *et al.,* 2019). Antibacterial activities were obtained by lemon peel extract with maximum inhibition zones of 10.67 ±3.09 mm against *Staphylococcus aureus* followed by *Bacillus subtilis* (9.20 ± 2.27mm), *Escherichia coli* (8.93 ± 3.08) whereas the minimum 8.00 ± 3.21mm inhibition zone was observed against *Pseudomonas aeruginosa*. The strongest antibacterial activities were obtained by tetracycline with maximum inhibition zone of 13.80 ± 4.59 mm against *Staphylococcus aureus* and minimum inhibition zone 10.73 ± 2.94mm against *Pseudomonas aeruginosa*. The negative control sample did not produce an inhibition zone (Table 4). Lemon peel extract indicated inhibitory effects on pathogens mentioned in this study which is in line with other several reports. Öntas *et al.* (2016) reported antimicrobial effect of lemon essential oil against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.* Hindi and Chabuck (2013) reported antimicrobial effect of different aqueous lemon extracts against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Streptococcus agalactiae* (6 Gram-positive) and *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhi*, *Proteus* spp., *Moraxella catarrhalis*, *Acinetobacter* spp. (8 Gram-negative) and *Candida albicans* isolates. In this study, the diameter of the inhibition zones formed in *Staphylococcus aureus* and *Bacillus subtilis* were

more significant than that of *Escherichia coli* and *Pseudomonas aeruginosa* due to differences in cell wall structure between the bacteria. *Escherichia coli* and *Pseudomonas aeruginosa* as gram-negative bacteria, have thick cell wall composed of lipoproteins, oligosaccharides, and phospholipids (Synowiec *et al.*, 2014). The presence of lipopolysaccharide as a cell wall coating causes inhibition of the diffusion of hydrophobic components in lemon peel extract, making it challenging to penetrate cell walls (Hosseini *et al.*, 2015). At the same time, *Staphylococcus aureus* and *Bacillus subtilis*, gram-positive bacteria, have more straightforward cell wall structure composed of peptidoglycan. This structure is easily penetrated by foreign compounds (Synowiec *et al.*, 2014).

Table 4 : Mean inhibition zones (mm) of Lemon peel extract

Bacteria	Lemon extract	Positive control (Tetracycline)	Negative control (Water)
E.c	8.93 ± 3.08^{c}	11.87 ± 3.02^{b}	0.00 ± 0.00^{a}
S.a	10.67 ± 3.09^{a}	13.80 ± 4.59^{c}	0.00 ± 0.00^{a}
B.s	9.20 ± 2.27^{a}	12.27 ± 3.73^{b}	0.00 ± 0.00^{a}
P.a	8.00 ± 2.21^{c}	10.73 ± 2.94^{a}	0.00 ± 0.00^{a}

Note: E.c, *Escherichia coli*; S.a, *Staphylococcus aureus*; B.s, *Bacillus subtilis;* and P.a, *Pseudomonas aeruginosa*
Means sharing different superscripts in the same column are significantly different ($p<0.05$)

The antibacterial activity of lemon peel extract according to concentrations is shown in Table 5. There was an increase in the inhibition zone's diameter as the concentration increased. The results of this study agree with the research conducted by Mallick *et al*. (2020) and Anandito *et al*. (2023). Also, Nadeem *et al.* (2022) showed that the increase in antioxidant activity along with the rise in the concentration of lemon basil leaf extract, was caused by the presence of phenolic compounds like flavonoids, alkaloids, and saponins in the basil leaf extract.

Table 5: Inhibitory zones (mm) of lemon peel extract at different concentrations

	Concentration(g/ml)				
Bacteria	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**
E.c	4.67 ± 0.58^{a}	7.00 ± 0.00^{b}	9.33 ± 0.58^{c}	10.33 ± 0.58^{c}	11.33 ± 0.58^{c}
S.a	6.67 ± 0.58^{a}	9.33 ± 1.53^{b}	9.67 ± 0.58^{b}	12.67 ± 0.58^{c}	15.00 ± 1.00^{c}
P.a	4.33 ± 0.58^{a}	4.67 ± 0.58^{a}	9.33 ± 0.58^{b}	9.33 ± 0.58^{b}	12.33 ± 1.58^{c}
B.s	5.67 ± 0.58^{a}	8.67 ± 1.53^{b}	9.67 ± 0.58^{b}	10.33 ± 0.58^{c}	13.67 ± 1.53^{c}

Note: E.c, *Escherichia coli*; S.a, Staphylococcus *aureus*; B.s, *Bacillus subtilis;* and P.a, *Pseudomonas aeruginosa*. Means sharing different superscripts in the same row are significantly different ($p<0.05$)

Conclusion

Fresh fish is highly perishable and even if stored in cold storage to prolong its shelf life, the processes are not sufficient to prevent bacterial growth. To improve the quality of fish, it is necessary to add preservatives to the fish properly during storage. Lemon peel extract used in this study, showed an antibacterial activity against fish bacterial pathogens and could be used as replacement to synthetic preservatives.

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Conflict of Interest

The authors declare no conflict of interest.

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