







## Research Article

# Investigation on Pomegranate (*Punica granatum*) Skin as a Potentially Effective Natural Food Preservative

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The study is aimed at estimating the possibility of using pomegranate peel extracts as a natural food preservative which was investigated in fish samples. All the extracts (cold water, hot water, and 70% methanol) have been tested against five bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella Typhi*, *Bacillus megaterium*, and *Bacillus subtilis*) using the agar disc diffusion method. Hot water extract showed the best result against *B. megaterium*. The zone of inhibition (ZOI) for hot water extract as crude was taken 20  $\mu$ l/disc was 25 mm. Total antioxidant activity and total phenolic content (TPC) were determined for different extracts. The results of a test for TPC showed that 70% methanolic extract (MeOH) and cold water extract, respectively, comprised 263.59 mg gallic acid equivalent (GAE)/g and 197.99 mg GAE/g of total phenolics. In addition, MeOH had a total antioxidant activity of 67.55  $\pm$  5.58 mg ascorbic acid equivalent (AAE)/g while hot water extract had a total antioxidant activity of 66.86  $\pm$  3.55 mg AAE/g. The extracts were applied to *Ompok pabda* fish, and the fish were preserved in prepared extracts for 24 hours in the open air. Total volatile nitrogen (TVN) and sensory parameters were evaluated at regular intervals of 0, 4, 12, 18, and 24 hours. The TVN value of the prepared extracts was found in order of hot water > MeOH > cold water. Fish preserved in hot water extract was in the acceptable range even after 24 hours. Methanol extract and hot water yielded superior outcomes in preserving the fish.

## 1. Introduction

Food preservation is a popular technique that has been used all over the world since ancient times. This helps by preventing the growth of fungus and bacteria [1]. The purpose of a preservative is to stop the harmful chemical and qualitative changes brought on by the natural flora of food degradation. Following the introduction of chemical preservatives, irradiation, and disinfection of storage and manufacturing materials, cold-air chilling, cold pickling, and deep freezing gained popularity at the turn of the century [2]. Mankind has utilized and been aware of natural food preservatives for a very long time. These are used in both raw and cooked foods to increase shelf life so that the flavor, aroma, texture, and actual food may be preserved for a longer amount of

time. Additionally, they stop food from degrading. There are two ways that a preservative can work: antibacterial and antioxidant. By fostering an environment unfriendly to them, antimicrobials prevent the growth of bacteria, yeast, mold, or fungi. Antioxidants prevent items from oxidizing, extending the shelf life, and improving the appearance of foods after harvest [3–5]. Artificial food preservatives are to blame for several health issues affecting the respiratory system, heart, blood, and other systems [6]. It has long been believed that artificial food color and food additives (AFCA) have an impact on children's behavior [7]. Natural food preservatives, on the other hand, are not significantly related to any significant health issues [8].

The fact that natural preservatives have always been in nature and are familiar to our immune systems leads many

individuals to believe that they are safer than artificial ones. Almost all plant-based preservatives are safe for people to consume [9]. There are, however, currently very few natural preservatives used in the food sector. In order to adhere to its microbiological safety regulations, the food sector has been under pressure to gradually phase out artificial preservatives and replace them with natural alternatives [10]. This led to a rise in the quest for new substitutes to be utilized in food preservation systems, such as alternative antimicrobial chemicals (with an ecstatic or caudal impact), a combination of conventional (used in low levels), and alternative antimicrobials [11].

Public awareness for using the natural preservatives in food has grown along with the social economy's quick expansion. In general, people will like foods without preservatives, but if these are not an option, they will pick foods with natural preservatives above those with synthetic ones. Natural preservatives ensure that the food is safe to consume and devoid of bacteria. Natural preservatives would preferably be safe, non-toxic, functional at low dosages, contribute no smell or color to food, have no medicinal uses, be label compatible, and, last but not least, be cost-effective. In general, microbes, animals, and plants are the three main sources of natural preservatives. Additionally, biologically active chemicals that are derived from algae, fungi, and other sources may be a source of fresh natural preservatives for the food sector [12]. The best approach to replacing synthetic food preservatives in part or entirely is to employ polyphenol-rich plants as natural alternatives. They are easily obtainable from natural sources. Because of their capacity to combat microorganisms and free radicals, as well as potential uses in the food industry, plant-based phenolic compounds—which are employed as organic food preservatives—are critical. However, it is still difficult to locate plant sources that are biologically active, affordable, and safe to use for food preservation. *Punica granatum* is one of the oldest and best-known fruits, and it has a reputation for being safe; therefore, we decided to study the effect of this plant extract on food preservation.

*Punica granatum* also known as pomegranate is a familiar fruit from ancient times. The pomegranate is native to Iran and Afghanistan, known in ancient Egypt. The antibacterial, antifungal, antiprotozoal, and antioxidant effects of pomegranate are outstanding [13, 14]. The edible part of the fruit is a rich source of saccharides, polyphenols, and important minerals. The biological significance of these compounds is immense due to their enormous antioxidant and antimicrobial properties [15]. So, the primary goal of the current study was to determine how to effectively use pomegranate extracts as natural preservatives. The purpose of this study was to evaluate whether extracts could be used commercially as effective natural preservatives in the fish firm.

## 2. Materials and Methods

**2.1. Collection of Plant Materials.** Pomegranate was collected from a well-known local fruit market near Dhaka City (Narsingdi District), Bangladesh. This pomegranate, which is grown primarily in Narsingdi, Sylhet, Chuadanga, and some other districts in Bangladesh, is a little bit smaller in

size. Following separation from the fruit, the peels were divided into smaller pieces and rinsed with distilled water. Then, it was exposed to the sun to dry. With the use of a mixer grinder, the dried peel was collected and ground before being kept in a dry place for later use. The research study utilized this plant sample in powder form (Figure 1).

**2.2. Proximate Analyses.** The proximate composition of the produced pomegranate powder, comprising moisture, ash, protein, fat, and fiber, was evaluated based on our previous work [16]. Evaporation weight loss at 105°C for 6 to 8 hours was used to calculate the moisture content. The amount of ash was measured using a muffle furnace that produced white ash after burning at 600°C for six hours. Using the nitrogen-to-protein conversion factor 6.25, the Kjeldahl method was used to determine the protein amount. Digestion, distillation, and titration were the three steps in the Kjeldahl method. By extracting the samples in hexane and analyzing the hexane residues left behind after evaporation, the amount of fat in the samples was measured. The crude fiber content was determined using fat-free samples that were then boiled in 200 ml (1.25%) sulphuric acid under reflux before being further filtered and rinsed with hot water to remove any remaining acidity. To render the sample non-alkaline, the residue was then boiled once more with 200 ml (1.25%) NaOH prior to filtering and rinsed with hot water. It was cooled and weighed. We burned the crude fiber for 20 minutes in a muffle furnace at roughly 600°C before cooling, weighing, and calculating the results. The carbohydrate content was ascertained using the calculated difference approach. By multiplying the proportions of protein, fat, and carbohydrates by the corresponding physiological energy values and adding the results, the energy value was determined.

**2.3. Preparation of Extracts.** Three types of extracts were prepared:

- (i) Cold aqueous extract: cold aqueous extract was prepared by soaking 25 g of the powder in 300 ml of distilled water for 7 days at 4°C
- (ii) Hot aqueous extract: the powder (25 g) was extracted with 300 ml distilled water in a Soxhlet apparatus for 12 h
- (iii) 70% methanol extract: the powder (25 g) was extracted with 300 ml 70% (v/v) methanol in a Soxhlet apparatus for 12 h

After that, all of the extracts were run through Whatman No. 1 filter paper and dried in a Memmert oven at 50°C for 4.0 hours at Dreieich, Germany. Until they were used, they were kept at 4°C in the refrigerator.

**2.4. Source of Microorganism.** The test bacterial pathogens included *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Salmonella Typhi* ATCC 6539, *Bacillus megaterium* ATCC 14581, and *Bacillus subtilis* ATCC 6633. All the bacterial and fungal pathogens were clinical isolates obtained from the food microbiology laboratory, IFST, BCSIR.

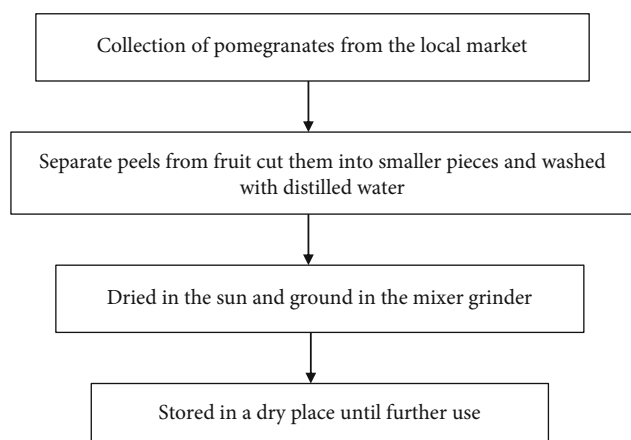


FIGURE 1: Preparation process of dried pomegranate peel powder.

**2.5. Assay of Antibacterial Activity.** The disc diffusion method was used in the current investigation to examine the antibacterial activity of all the crude extracts [17]. Discs containing 20  $\mu$ l of test material were seeded equally with the test microorganisms on a nutrient agar medium. As a positive and negative control, blank discs (impregnated with solvents) were employed. To allow for maximal diffusion, the plates were held at a low temperature (4°C) for 24 hours. The dried disc began to absorb water from the environment at this time. The test substances were then released from the sample disc by dissolving and diffusing. According to the physical law that governs the diffusion of molecules through agar gel, diffusion takes place. As a result, the concentration of the test elements in the media surrounding the discs gradually changed. To allow the microorganism to grow as much as possible, the plates were incubated at 37°C for 24 hours. A clear, unambiguous zone of inhibition could be seen around the medium. The diameter of the zone of inhibition (ZOI), which is stated in millimeters, was used to calculate the test agent's antimicrobial activity. Each step of the test was repeated three times.

**2.6. Total Antioxidant Assay (TAA).** The phosphomolybdenum assay was used to measure the total antioxidant capabilities of the aqueous and methanolic extracts, which is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent development of a green phosphate-Mo (V) complex in acidic conditions. 1 ml of reagent solution containing 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate was mixed with 0.1 ml of each extract [18]. A boiling water bath was used to incubate the reaction mixture for 90 minutes at 95°C. A UV-visible spectrophotometer was used to measure the absorbance of the solution at 695 nm after it had cooled to room temperature. The blank was 0.1 ml of methanol. The quantity of milligram equivalents of ascorbic acid per milliliter of extract was used to assess the total antioxidant capacity.

**2.7. Total Phenolic Content (TPC).** The TPC of the extract was ascertained using the modified Folin-Ciocalteu method [19]. TPCs were calculated using this approach, which used

gallic acid as the reference. 1 ml solution of the plant extracts or standard with different concentrations was mixed with other chemical reagents, including 5 ml Folin-Ciocalteu reagent (Merck, Germany) (previously diluted with water 1 : 10 v/v) and 4 ml (7.5% sodium carbonate) of sodium carbonate. For color development, the tubes were vortexed for 30 seconds and let to stand at 20°C for 30 minutes. Using a spectrophotometer (Shimadzu UV/Visible Scanning Spectrophotometer 1800, Japan), the absorbance of the samples and the standard was measured at 765 nm against a blank. The solvent (methanol, water) used to dissolve the plant extract was present in a standard blank solution.

The following equation was used to measure the total content of phenolic compounds in plant extracts in gallic acid equivalents (GAE):

$$C = \frac{c \times V}{m}, \quad (1)$$

where  $C$  is the total content of phenolic compounds in GAE (mg/g plant extract),  $c$  is the concentration of gallic acid established from the calibration curve (mg/ml),  $V$  is the volume of extract in milliliter, and  $m$  is the weight of crude plant extract in gram.

**2.8. Technological Application.** Determining a fresh fish's shelf life, also known as storage life, or how long it will remain edible, is related to evaluating its quality. There are two ways to assess the quality of fish to estimate its freshness and shelf life. Both sensory and nonsensory methods are used here. While nonsensory approaches use physical, biochemical, chemical, and microbiological means, sensory methods primarily depend on the fresh fish, fruit, and vegetable's look, odor, texture, and taste to determine if they should be accepted or rejected [20]. A variety of biochemical techniques have been proposed and developed to assess one or more quality indices and utilize the results as a symptom of fish deterioration. The quality and increasing deterioration in the shelf life of collected fish samples maintained in the open air were evaluated using the biochemical total volatile nitrogen (TVN) test, which is the standard test for the freshness of fish, fruit, and vegetables.

**2.8.1. Preservation of Ompok Pabda Fish.** Fresh *Ompok pabda* fishes were collected from a local market to use as a control sample. Extracts were dissolved in water at a concentration of 10 mg/ml. Fishes were submerged in prepared extract solution and kept for 2 hours at room temperature. Fish samples were then preserved at room temperature overnight. Sensory parameters such as color, odor, texture, appearance, and total volatile nitrogen were determined for the samples afterward.

**2.8.2. TVN Determination.** TVN was determined by Pearson's method [21]. 25 ml of 10% trichloroacetic acid (TCA) was added to 2 g of ground fish kept overnight and titrated with a known volume of boric acid. TCA,  $K_2CO_3$ , and the solution made from the fish samples were taken into the Conway dishes in the following way. Each dish was then covered by a piece of glass that had been initially stickled

with glue (paraffin soft white). Then, it was kept for 24 hours and titrated with N/70 H<sub>2</sub>SO<sub>4</sub>. Finally, TVN was calculated.

$$\begin{aligned} \text{TVN} = & (\text{titration reading} - \text{blank reading}) \\ & \times \text{strength of acid} \times 0.2 \\ & \times \frac{\text{volume of the extract}}{\text{volume of the extract taken}} \\ & \times \frac{100}{\text{weight of the sample taken}} \end{aligned} \quad (2)$$

TVN in the *Ompok pabda* fish samples in different sample extracts (cold water, MeOH, and hot water) was measured and then kept in the open air. Variations in the rate of changes of TVN were recorded.

**2.8.3. Sensory Analysis.** The assessment was laid on skin appearance, texture, odor, discoloration, and overall acceptability of the fish. Samples were presented in front of eight trained panelists. The scores on the hedonic scale ranged from 1 to 5 as 5 = highly acceptable, 4 = very much acceptable, 3 = moderately acceptable, 2.5 = borderline of acceptability, 2 = slightly unacceptable, and 1 = very much unacceptable. The higher score values indicate a greater preference.

**2.9. Statistical Analysis.** All data are presented as mean values ( $\pm$ S. E). Analysis of variance (ANOVA) was performed using IBM SPSS 20.0 for Windows 7 where a statistically significant difference was determined at  $p < 0.05$ , the means were further separated using Duncan's multiple range test. In each experiment, three replicates were performed.

### 3. Results and Discussions

**3.1. Proximate Analysis.** The average percentages of ash, moisture, fat, carbohydrate, and protein were calculated and are shown in Table 1. As shown in Table 1, the moisture content of produced pomegranate fruit peel was found to be  $14.46 \pm 2.42\%$ . In addition, crude protein, fat, ash, crude fibers, carbohydrates, and pH contents for pomegranate fruit peel powder were  $8.23 \pm 0.34$ ,  $4.04 \pm 0.54$ ,  $2.41 \pm 0.03$ ,  $7.75 \pm 0.09$ ,  $63.11 \pm 3.43\%$ , and  $6.74 \pm 0.01$ , respectively. Pomegranate fruit peel had 376 kcal/100 g (dry basis) of energy. With some changes, prior investigations revealed generally consistent results. The pomegranate fruit peel, for example, was discovered by Omer et al. to have 6.52% protein and 3.43% ash [22]. However, they discovered that the pomegranate fruit peel had a slightly greater energy level (428 kcal/100 g) and ash content but a lower protein content [22]. In another study, Ismail et al. found that the pomegranate peel contains a similar amount of ash, less fat and protein, and greater amounts of fiber and carbohydrates [23]. Overall, this study indicated that the nutritional content may vary slightly depending on the local or regional context.

**3.2. Antimicrobial Screening.** Three out of five bacteria used (*Escherichia coli*, *Staphylococcus aureus*, and *Salmonella Typhi*) are Gram-negative, and two bacteria (*Bacillus megaterium*, *Bacillus subtilis*) are Gram-positive. There was

TABLE 1: Proximate composition of pomegranate peels (per 100 g portion).

Components	Composition (means $\pm$ SD)
Carbohydrate	63.11 $\pm$ 3.43
Protein	8.23 $\pm$ 0.34
Fat	4.04 $\pm$ 0.54
Crude fiber	7.75 $\pm$ 0.09
Moisture	14.46 $\pm$ 2.42
Ash	2.41 $\pm$ 0.03
pH	6.74 $\pm$ 0.01

significant variation in the zone of inhibition of different extracts (Table 2). For *E. coli*, the zones of inhibition values of three extracts (cold water, methanol, and hot water) were between 18 and 24 mm. The maximum inhibitory effect was recorded by methanol extract; however, the cold water extract had very little inhibitory effect. The effect of cold water extracts was less on *S. aureus* compared to methanolic extracts where an antibacterial effect was observed with a zone of inhibition value of 21.5 mm which was the highest for this bacterium. The zone of inhibition observed for hot water extract was 21 mm. For *S. Typhi*, the inhibitory effects were recorded by extracts of the pomegranate peel. The zone of inhibition value for cold water was 9 mm. The maximum inhibitory effect was shown by methanol extract which was 12 mm. In the case of *B. megaterium*, the zone of inhibition values was between 25 and 13 mm, all three extracts were inhibitory for this bacterium, the high inhibitory effect was exhibited by hot water extract followed by cold water extract, and the lowest effect was exhibited by methanol extract. Comparatively, the effect of methanol extract was high on *B. subtilis* with the zone of inhibition value between 13.0 mm. The inhibitory effect observed with cold and hot water extract was similar to the zone of inhibition value of 11 mm. Pomegranate fruit peel extract has been shown in prior research to reduce microbial activity. Pomegranate peels, which are rich in antioxidants and broad-spectrum antibacterial agents and can even stop food from spoiling, are the main by-products produced during food processing of pomegranate fruits. The types, growing circumstances, cultivation tactics, developmental stages, and extraction techniques are all recognized to have a substantial impact on the pomegranate's health potential [24]. Alexandre et al. stated that pomegranate peel extracts (PPE) could be employed as a source of bioactive chemicals with a high added value for applications such as antibacterial and antioxidant protection [25]. Pomegranate extracts are potential sources of organic, plant-derived antimicrobials that can be used instead of synthetic antimicrobials [26]. All things considered, pomegranate extracts might be used as natural preservatives for foods like fish.

**3.3. Total Phenolic Content (TPC).** One of the most important classes of molecules that serve as primary antioxidants, notably free radical terminators, is phenolic components [27]. Table 3 displayed the TPC for the selected pomegranate peel

TABLE 2: Antimicrobial effect of pomegranate peel extracts.

Sl	Samples	Zone of inhibition (mm)				
		<i>E. coli</i>	<i>S. aureus</i>	<i>S. Typhi</i>	<i>B. megaterium</i>	<i>B. subtilis</i>
1	P1	18.0	20.5	9.0	22.5	11.0
2	P2	24.0	21.5	12.0	22.0	13.0
3	P3	20.0	21.0	11.0	25.0	11.0

extracts that were evaluated. 70% MeOH extract showed high-value contenance of gallic acid. Cold water extract and hot water extract also showed high contenance of GA. The phenolic compound content in 70% MeOH extract was  $263.59 \pm 10.8$  mg GAE/g. From cold water extract, obtained phenolic compound was  $197.99 \pm 8.7$  mg GAE/g. Hot water extract contains phenolic compound  $178.61 \pm 7.5$  mg GAE/g. Consuming fruits and vegetables with high phenolic content has been associated with a lower death risk from cancer, cardiovascular disease, and cerebrovascular illnesses, according to epidemiology research [28]. Pomegranate phenolic compounds may have advantageous effects via scavenging free radicals [29]. The concentrations of phenolic compounds in the pomegranate fruit's arils and peel changed throughout storage, and this study demonstrates how the abundance of particular chemicals is related to the activity of antioxidant enzymes. The utilization of pomegranate resources and the development of suitable postharvest procedures to preserve food quality would benefit from this knowledge [30]. Phenolic compounds will also be helpful for the inhibition of the oxidation process initiated in food. Phenolic compounds can increase the shelf life of food and foodstuffs. It also assessed how using pomegranate peel affects the flavor and oxidative stability of meat products. Pomegranate peel phenolics, which act as a natural preservative, may enhance the quality of meat products that have been preserved, preserving the color, restricting the growth of microorganisms, and delaying the oxidation of lipids and proteins [31]. All things considered, it suggests that the phenolic compounds found in pomegranates may be associated with food storage.

**3.4. Total Antioxidant Activity.** Total antioxidant activity was found in higher amounts for 70% MeOH extract, cold water, and hot water extract. Among these extracts, 70% MeOH extract showed the highest antioxidant activity. The total antioxidant activity of 70% MeOH extract was  $67.55 \pm 5.58$  mg AAE/g. The total antioxidant activity of hot water and cold water extract was  $66.86 \pm 3.55$  mg AAE/g and  $61.4 \pm 5.05$  mg AAE/g, respectively. Based on reports, pomegranate juice has a higher total antioxidant capacity than other popular drinks. According to the many evaluation techniques, pomegranates have a high overall antioxidant capacity, albeit there can be differences depending on the cultivar, region of origin, processing, and other elements [32]. Furthermore, pomegranate peel was shown to have significantly more total phenolics, flavonoids, and flavonols than the fruit's seeds and drinks. It was shown that there was a strong positive association between total phenolic content and antioxidant activity. The high antioxidant capacity of pomegranate, particularly the peel, has made it possible to employ them as natural

food preservatives, according to the findings [33]. The current study shows that pomegranate fruits are a high dietary antioxidant source and that they are capable of being employed as natural food preservatives.

**3.5. TVN Determination.** The collective measurement of TVN can be the parameter for estimating the microbial deterioration of fish. Several volatile bases are released in fish during decomposition by bacteria. The increase in TVN might be due to microbial activity under low temperatures. Being a perishable product, meat or fish undergoes chemical compositional changes during storage as a result of the operations of microbes and endogenous enzymes. A common indicator of protein and amine breakdown is TVN [34]. TVN value was accepted till 30 mg/100 g in raw fish and was compared with the control, while the TVN value of 50-70 mg/100 g of muscle can be considered the highest limit beyond which fish is considered inedible. For salted dried fish, the range is 100-200 mg/100 g beyond which products are marked unacceptable. Pabda fish preserved in distilled water was taken as control. TVN was determined after the preservation of pabda fish using extracts of pomegranate. Fishes were preserved 24 hours at room temperature, and fish samples were collected for TVN determination after different time intervals. Variations in the rate of changes in TVN are shown in Table 4. The initial TVN of the collected sample was  $9.48 \pm 0.05$  mg/100 g. The change in TVN was determined after for 0 hours (initial time), 4 hours, 12 hours, 18 hours, and 24 hours. The change in TVN for the control was  $13.07 \pm 0.10$ ,  $13.23 \pm 0.09$ ,  $20.26 \pm 0.365$ ,  $29.10 \pm 0.68$ , and  $47.32 \pm 1.06$  mg/100 g for 0 hours (initial time), 4 hours, 12 hours, 18 hours, and 24 hours, respectively. After 18 hours, TVN value of control reached an unacceptable range. Hot water and methanol extract showed better results. Fish preserved in hot water extract was in the acceptable range even after 24 hours. TVN value for cold water and 70% MeOH extract was still in the acceptable range after 24 hours, but the value of TVN was lower than the value observed for hot water extract. The TVN value of the prepared extracts was in the order of hot water > MeOH > cold water. Measures of color, microbial count, softness, and moisture content have a relationship to TVN. This makes sense given that fresh meat or fish is related to low TVN concentrations, as well as higher color values, water-holding capabilities, low microbial counts, and low levels of proteolysis. Therefore, it can be said that TVN offers useful and trustworthy knowledge about the level of spoiling meat or fish [35]. The total volatile basic nitrogen may be greatly reduced by treating the shrimp with a pomegranate peel extract solution at both levels before and after storage. On

TABLE 3: Effect of pomegranate peel extracts on total phenolic content (TPC) and total antioxidant activity (TAA).

Sample extracts	TPC (mg GAE/g)	TAA (mg AAE/g)
Cold water extract	197.99 ± 8.7 <sup>b</sup> mg GAE/g	59.4 ± 2.05 <sup>b</sup> mg AAE/g
MeOH extract	263.59 ± 10.8 <sup>a</sup> mg GAE/g	67.55 ± 5.58 <sup>a</sup> mg AAE/g
Hot water extract	178.61 ± 7.5 <sup>c</sup> mg GAE/g	66.86 ± 3.55 <sup>a</sup> mg AAE/g

The values represent mean ± SD. Three replicates were used in each experiment. Means with different superscripts across rows (lowercase alphabets) indicate significant differences ( $p < 0.05$ ).

TABLE 4: Effect of pomegranate peel extract on TVN values (mg/100 g) of *Ompok pabda* fish at different time intervals (mean ± SD).

Sl	Sample	0 hours (initial time)	4 hours	12 hours	18 hours	24 hours	Acceptable limit
1	Control	13.07 ± 0.10 <sup>a</sup>	13.23 ± 0.09 <sup>a</sup>	20.26 ± 0.36 <sup>a</sup>	29.10 ± 0.68 <sup>a</sup>	47.32 ± 1.06 <sup>a</sup>	
2	P1	10.03 ± 0.05 <sup>d</sup>	10.11 ± 0.07 <sup>d</sup>	17.28 ± 0.13 <sup>b</sup>	22.06 ± 0.42 <sup>c</sup>	28.57 ± 0.18 <sup>c</sup>	
3	P2	11.34 ± 0.06 <sup>c</sup>	11.39 ± 0.04 <sup>c</sup>	13.71 ± 0.04 <sup>d</sup>	22.56 ± 0.03 <sup>c</sup>	27.12 ± 0.12 <sup>d</sup>	
4	P3	12.30 ± 0.14 <sup>b</sup>	12.51 ± 0.43 <sup>b</sup>	15.49 ± 0.31 <sup>c</sup>	26.42 ± 0.33 <sup>b</sup>	30.56 ± 0.03 <sup>b</sup>	<30.00

P1 = sample preserved in cold water extract; P2 = sample preserved in 70% MeOH extract; P3 = hot water extract. Mean values ± standard error ( $n = 3$ ). Means within a row followed by different letters are significantly different ( $p < 0.05$ ).

TABLE 5: Sensory analysis of the preserved fish using pomegranate peel extract after 24 hours.

Sl no.	Condition	Storage hours	Skin appearance	Texture	Odor	Discoloration	Sensory score
1	Control	4	5	5	4	5	4
		12	4	3	3	3	3
		18	2.5	2	2	2.5	2
		24	2	1	1	2	1
2	P1 treated	4	5	5	5	5	5
		12	4	4.5	4.5	5	4.5
		18	3	4	3	3	3
		24	2	2	2	2.5	2
3	P2 treated	4	5	5	5	4.5	5
		12	4.5	4	4.5	3.5	4
		18	4	3	4	3	3
		24	3	2.5	3	2.5	2.5
4	P3 treated	4	4.5	4	4.5	4.5	4.5
		12	3.5	3	3	2.5	3
		18	2.5	2.5	2.5	2	2.5
		24	2	1	2	2	2

5 = highly acceptable; 4 = very much acceptable; 3 = moderately acceptable; 2.5 = borderline of acceptability; 2 = slightly unacceptable; 1 = very much unacceptable.

day 6, shrimp treated with pomegranate peel extract had a lower melanosis score, but on day 10, they had higher sensory scores for odor, texture, color, and overall likeness [36]. Pomegranate waste extract significantly reduced total volatile nitrogen (TVN), total bacterial counts, *Staphylococcus aureus*, coliforms, and *Escherichia coli* during days 6 and 9 of the storage period for poultry carcasses [37]. Pomegranate extract application could be a natural, secure, and safe decontamination intervention in an integrated food safety system.

**3.6. Sensory Analysis.** Table 5 illustrates the sensory analysis of *Ompok pabda* (control, cold water, 70% MeOH, and hot

water extracts) at zero time and after storage for 24 hours at room temperature. As shown from this table, treatment with hot water extract significantly enhanced all the sensory characteristics of *pabda* fish. In sensory analysis, the skin appearance and color decreased in a time-dependent manner, the texture of the body was loosed, and the odor was increased due to increment of storage period at room temperature (26°C). The results reveal that all the samples were up to the standard value at the 0th hour. Sensory characters change over time for both control and samples. After 24 hours, sensory characters reduced quality to a considerable level for control and samples treated with extracts. Fish treated with 70% MeOH extract scored highest among all



FIGURE 2: *Ompok pabda* fish after a 24-hour preservation period.

the samples. The score showed that overall acceptance of the fish sample was highest for 70% MeOH during the 18th hour. The color of all the fish samples turned slightly yellowish after 24 hours (Figure 2). The overall acceptance was significantly high for fish treated with 70% MeOH extract. Samples treated with cold water extract did not show good results after 24 hrs. Skin appearance, texture, odor, discoloration score, and overall acceptance score were slightly higher than the results of control fish samples. Similarly, hot water extract also showed a decrease in sensory score with the increase of time. After 24 hours, fish samples treated with hot water extract also showed a decrease in sensory score. Pomegranate peel extracts contain antioxidants that stop the growth of peroxide molecules which maybe they have a preservation impact on fish [38, 39]. It was mentioned that the sensory score decreased with the increment of the TVN value of the fish products [40]. The pomegranate peel extract solution is thought to improve sensory evaluations of shrimp's odor, texture, color, and overall likeness [36]. When compared to untreated carcasses during adequate shelf life, the sensory qualities in pomegranate extract-treated carcasses have been greatly improved [37]. Altogether, the application of pomegranate extract may be a natural, secure, and safe decontamination strategy in a comprehensive food preservation system.

#### 4. Conclusion

Pomegranate peel extracts (PPE) have substantial antibacterial and antioxidant properties that make them useful for food preservation. The use of PPE extends the shelf life of perishable foods by effectively combating a range of microorganisms that lead to food spoilage. Pabda fish samples are an excellent representation of how food products may be made more stable and storage-friendly without sacrificing their sensory qualities. This implies that PPE presents an invaluable and inexpensive natural substitute for synthetic preservatives. It could benefit the food industry and other industries that produce and store consumable goods by addressing the health and environmental concerns raised

by synthetic preservatives. The optimization of PPE concentration and formulation, compatibility with various foods, and the underlying mechanisms of its antibacterial and antioxidant capabilities should all be the subject of future study. As scientific understanding evolves, the application of PPE to a wide range of sectors may offer a long-term method of extending the shelf life of food that is perishable without reducing its sensory appeal or overall quality.

#### Data Availability

All data are provided inside the manuscript.

#### Conflicts of Interest

There are no reported conflicts of interest for the authors.

#### Authors' Contributions

The research was equally contributed by Mohammad Lokman Hossain and Md. Nazim Uddin. The study originated and is under the direction of Mohammad Lokman Hossain, Md. Zahurul Haque, and Shamsun Naher. Mohajira Begum and Tania Nowreen Orchy performed the experiments and designed the illustration for this publication. The English language in this text was written, revised, and edited by Md. Nazim Uddin and Tania Nowreen Orchy.

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