Extracting, evaluating biological activities of phenolic compounds from yellow onion peels (*Allium cepa* L.) and their applicability for fish preservation.

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**Abstract.** The research aimed to evaluate total phenolic content extracted from onion (*Allium cepa* L.) peels and some of the extract biological activities. The potential of the extract for preserving fish was also investigated. Onion peel extracted with EtOH 60% at 60°C (60 minutes, solid/liquid ratio 1g/20ml) yielded highest polyphenol content 168.49 ± 0.02 GAE/g DW and flavonoid content (216.43 ± 0.06 mg QE/g DW). The extract showed potent antioxidant property in DPPH radical scavenging assay with IC$_{50}$ value of 12.48 ± 0.45 µg/mL and good antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, *Pseudomonas aeruginosa* with MIC values of 175; 175; 200; 200; 150 (µg/ mL) respectively. The extract also displayed alpha-glucosidase inhibition activity with IC$_{50}$ value of 142.53 µg / mL and negligible tyrosinase inhibition activity. Measurement of the PV, TBARS values of basa catfish fillets coated with the extract solution at different concentrations, it was observed that fish samples treated with 3% extract solution could greatly reduce the lipid oxidation under 4°C and -18°C storage conditions compared with untreated samples. The PV, TBARS values after 4 weeks were comparable to 200ppm BHT treated samples.

1. **Introduction**

Onion (*Allium cepa* L) is the second most consumed vegetable worldwide as a source of nutritious food and spice [1]. Diets high of onions are reported to support health promotion and decrease the risk of cancers, vascular and heart diseases, neurodegenerative disorders and cataract formation [2]. Their significance is related to their high contents of health benefit phytochemicals, of which organosulfur, polyphenol, flavonoid compounds are among major contributors [3]. Onions are the richest source of human diet flavonoids [4]. High levels of flavonoids have been claimed to be the main factor responsible for onion’s strong antioxidant and a variety of pharmacological activities [3],[5]. The primary flavonoids found in onions are quercetin-3,4’-O-diglucoside (QDG) and quercetin-4’-O-monoglucoside (QMG), which make up around 90% of the total flavonoid content [6-8]. Quercetin has long been recognized as a powerful flavonol possessing multiple important therapeutic properties, including potent antioxidant, anti-inflammatory, anti-diabetic, antitumor and also broad-spectrum antimicrobial activities [9-11]. Interestingly, many studies demonstrated that total flavonoid and quercetin content in the dry outer skins is significantly higher than that in the edible parts and a
decrease is found from outer to inner parts on both a fresh and dry weight basis. The content of phenolics extracted from onion skins was nearly six times higher than that of their flesh counterparts [13]. A study on red onions observed that the amounts of isolated phenolic and quercetin compounds from the onion skin were approximately 3 to 5 times higher as from the flesh part. The skin extract exhibits antioxidant and radical scavenging activities comparable to that of butylated hydroxytoluene (BHT) and high antimicrobial activity against Escherichia coli, Pseudomonas fluorescens and Bacillus cereus and fungi Aspergillus niger, Trichoderma viride and Penicillium cyclopium, while edible part extract showed lower to no effects against tested microorganisms [14]. In another work, subcritical-water extracts (SWE) of orange onion peels demonstrated approximately 4-fold DPPH scavenging effects that of BHT. The extract was more effective than BHT at 61.3 ppm in lipid peroxidation inhibitory effects. In addition, the antioxidative activity of SWE extract measured via the ferric thiocyanate (FTC) method was 2-fold that of BHT [15]. Quercetin and quercetin glycoside fractions isolated from yellow onion skins were also exhibited potent radical scavenging activity against DPPH, superoxide anion (O2−), and hydroxyl (·OH) radicals [16]. Screening of antibacterial activity against two MRSA strains and two H. pylori strains of isolated products obtained from a water extract of yellow onion, it was found that compound (4) 3-(quercetin- 8-yl)-2,3-epoxyflavanone, a quercetin-derived oxidation product, was more potent than quercetin against MRSA (inhibition zone 15-18mm, while that of quercetin was 10-11mm) and both had strong effects against H. pylori. Oxidative quercetin derivatives were also 2-2.5 folds more active than quercetin in DPPH scavenging test [17]. Moreover, it was reported that long-term intake of quercetin–rich onion peel extract (OPE) may bring benefits by its anti-thrombotic effects through restricting the induced expression of tissue factor via down-regulating mitogen-activated protein kinase (MAPK) activation upon coagulation stimulus, leading to the prolongation of time for arterial thrombosis [18]. Noticeably, in a recently published paper, Allium Cepa L. peel extracts were proved to possess promising anti proliferative activities on three cancer cell lines: Hela (cervical cancer cell line), HCT116 (human colon cancer cell line) and U2OS (human osteosarcoma cell line) [19].

Despite considerable levels of flavonols and many proved beneficial activities, the outer scale of onion is practically peeled off and discarded as food wastes. This study examined the extracting conditions to obtain rich polyphenol, flavonoid extracts from yellow onion skins. The antioxidant, antimicrobial, alpha-glucosidase inhibition and tyrosinase inhibition properties of the extract were evaluated. The application of the extract as fish preservative agents was also investigated. The research results hope to provide insights into the value and the possibility of this onion waste to be exploited in food industries.

2. Materials and Methods

2.1. Materials

Yellow onion peels were collected from local markets. Folin and Ciocalteau’s phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, quercetin, tyrosinase enzyme, α-glucosidase enzyme, L-DOPA, para-nitrophenol, Kojic acid, 1,1,3,3-tetra methoxypropane (TMP), BHA (3-tert-butyl-4-hydroxyanisole) were purchased from Sigma–Aldrich. Bacteria Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Salmonella, Pseudomonas aeruginosa was provided by Vietnam Type Culture Collection.

Onion peels were removed impurities and damaged pieces and washed with water before being dried under a ventilated condition at 37°C. Then, the dry peels were cut, ground into small pieces of about 1-3 mm to be used as raw materials for extraction.

Live basa catfish (Pangasius bocourti) was obtained from the local market.

2.2. Extraction of Phenolic Compounds.

Onion peels (2 g) were extracted with ethanol-water with continuous stirring. Different extracting parameters were examined (temperature, ethanol concentration, time, solid/liquid ratio). After
extraction, the mixture was filtered and total phenolic content and total flavonoid content of the extract were determined to compare extraction efficiency and find the best condition. All extractions were performed in triplicate and the results were expressed as means ± standard deviation (SD).

Figure 1. Dried onion skin and the extract solution

2.3. Measurement of Total Phenolic Content.
The total phenolic content of the extracts was determined using Folin-Ciocalteu assay [20]. All extracts were diluted 1:100 with distilled water to obtain readings within the standard curve ranges of 0-150 ppm gallic acid. The diluted extracts (100 µL, two replicates) were introduced into test tubes; 500 µL of Folin-Ciocalteu’s reagent and 400 µL of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 60 min. Absorption at 760 nm was measured. The total phenolic content was calculated as gallic acid equivalents (GAE) in milligrams per gram dry weight of onion peel using the gallic acid calibration curve.

2.4. Measurement of Total Flavonoid Content
The total flavonoid content of the extract was measured by the aluminium chloride colorimetric assay with slight modification [21]. Briefly, 1 mL of 1:10 diluted extracts or standard solution of quercetin was mixed with 4 mL of distilled water, and subsequently with 0.3 mL of 5% sodium nitrite solution. After 5 minutes, 0.3 mL of 10% aluminium chloride was added and allowed to further react for 5 min before 2 mL of 1 M sodium hydroxide and distilled water were added to bring the final volume of the mixture to 10mL. The solution was mixed well, and the absorbance was immediately measured against a prepared blank at 510 nm wavelength using a UV spectrophotometer. The flavonoid content was determined by a quercetin standard curve and expressed as milligrams of quercetin equivalents (QE) / g of dry material.

2.5. Measurement of DPPH radical scavenging capacity
The DPPH assay was performed according to the methodology described by Sharma et al. [22]. 120 ml of extract solution of different concentrations in methanol was mixed with 180 ml of 6mM DPPH in methanol and shook well. After 30 minutes of incubation at room temperature in the dark, the reaction mixture absorbance was measured at 517 nm. The percentage of DPPH inhibition was calculated as follow:

\[
\text{% inhibition} = \frac{(Ac - As)}{Ac} \times 100
\]

Ac: absorbance of the control (DPPH + methanol), As: the absorbance of the reaction mixture.
The inhibitory concentration of the extract solution needed to inhibit 50% of the DPPH radicals obtained from the standard curve (IC\(_{50}\)) was compared to that of BHT as antioxidant reference standard.

2.6. Measurement of tyrosinase inhibition property
Tyrosinase inhibition was determined using the modified dopachrome method with L-DOPA as a substrate [23]. Assays were conducted in a 96-well microtiter plate and a plate reader was used to measure absorbance at 475 nm. Each test sample (well C) contained 40 µl extract solution of different concentrations in 5% DMSO, 40 µl tyrosinase solution (138 U/ml in 0.1M phosphate buffer pH 6.8).
and 100μl substrate solution (2.5 mM L-dopa in 0.1M phosphate buffer pH 6.8). Each test sample was accompanied by a blank sample (well D) that had all the components except tyrosinase. Each test or blank sample was compared with a control sample consisting of 5% DMSO solution in place of extract solution (well A with tyrosinase, well B without tyrosinase). The assay mixture was incubated at room temperature for 7 minutes, and the absorbance at 475 nm was measured. Kojic acid was tested as a positive control sample. The percentage tyrosinase inhibition was calculated as follows:

\[
\% \text{ inhibition} = \frac{(A - B) - (C - D)}{(A - B)} \times 100
\]

Where, A, B, C, D = OD of well A, B, C, D.

2.7. α-glucosidase inhibitory activity

The assay was carried out according to the standard method with minor modification [24]. Assays were conducted in a 96-well microtiter plate and a plate reader was used to measure absorbance at 405 nm. Test samples containing 100 μl phosphate buffer (0.1 M, pH 6.8), 20 μl α-glucosidase (1 U/ml), and 40 μl of varying concentrations of extract in 5% DMSO were preincubated at 37°C for 5 minutes. Then, 40 μl para-nitrophenyl α-D-glucopyranoside (5 mM) was added as a substrate and incubated further at 37°C for 30 min. The reaction was stopped by adding 100 μl Na2CO3 (2 M). The absorbance of the released p-nitrophenol was measured at 405 nm using a multiplate reader. Acarbose at various concentrations (0.1–0.5 mg/ml) was included as a standard. A control sample without the extract was set up in parallel with each test sample and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula,

\[
\text{Inhibitory activity (\%)} = (1 - \frac{A_s}{A_c}) \times 100
\]

Where, As is the absorbance of the test sample and Ac is the absorbance of the control sample.

2.8. Antibacterial assays

Evaluation of antibacterial susceptibility of the onion peel extract was determined based on the Kirby - Bauer disk diffusion method [25]. The extract solution in ethanol was injected into an agar hole with a diameter of 6 mm, then the solution was diffused into the agar layer to inhibit the growth of bacteria in the surrounding area. The larger the inhibition zone, the greater the antibacterial effect of the extract. Five strains Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Salmonella, Pseudomonas aeruginosa were grown for 24h in NB medium (peptone, 5 g/L; yeast extract, 10 g/L; NaCl, 5 g/L). After incubation, each 50μL bacterial suspension, at a concentration of 106 cells/mL, was spread over the surface of NA medium (NB added 20 g/L agar) plate and the plates then was left to dry for five minutes. Wells were made in the agar using a sterile cork borer and the disc was punched with 5 holes 6 mm in diameter. Precisely 50 μL of the test solution was introduced into each agar hole. The plate were allowed to stand in the refrigerator for one hour and then incubated at 37°C. Zones of growth inhibition were measured after 16 hours of incubation. Each microorganism was tested in triplicate and the solvent (ethanol) was used as a control.

Minimum Inhibitory Concentration Determination

The antibacterial activity of the extract was investigated at different concentrations to determine MIC value, which was defined as the lowest concentration of the extract solution at which no bacterial growth was observed.

2.9. Lipid oxidation inhibition on Yellowtail Catfish fillets during storage

Live basa catfish after cleaning, removing skin was cut into fillets of 5 × 5 × 2 (cm³). The extract suspension was prepared as follows: x milligram of concentrated extract (moisture content <10%) was dissolved in 1ml of ethanol, then 19ml of distilled water was added with stirring thoroughly to make a fine suspension of different concentrations 0.5-5% (w/w of dried weight extract/the suspension). The fillet was dipped in the extract suspension for 30 minutes. After that, it was left dried in the air for five
minutes before putting in a zipping plastic bag for storage. The storage conditions examined were cold (4°C) and freezing (-18°C).

2.9.1. Peroxide Value measurement (PV)
PV was determined according to Cox and Pearson [26]. To a flask containing 1g of the fillet was added 5 ml of chloroform and 10 ml acetic acid and the mixture was shaken well. To initiate the reaction, 1 mL of saturated potassium iodide (KI) was added and shaken thoroughly in 1 minute. After 5 minutes left in the dark, 75 mL of distilled water was added and mixed. The peroxides oxidized the iodide to iodine, and the iodine was titrated to a colorimetric endpoint (blue color disappeared) using 0.01 N sodium thiosulfate (Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3}) solution with starch (10%) as an indicator. The amount of produced iodine was directly proportional to PV. The peroxide value (PV) was expressed in milliequivalents of active oxygen per one kilogram (mEq/kg) of processed fish sample, as determined by employing the following formula

\[ PV = \frac{(V - V_0).N.1000}{m_0} \]

With V, V\textsubscript{0}; volume of sodium thiosulfate solution used for the test sample and the blank (ml), respectively; N: represent the molarity of sodium thiosulfate solution; m\textsubscript{0}: mass of the test sample (g)

2.9.2. TBARS (Thiobarbituric acid reactive substances) assay: TBARS was determined according to the method of Buege and Aust [27]. The fish sample (3 g) was grinded with 15 ml of distilled water in and the mixture was centrifuged. 2 ml of solution from the centrifuge tube was transferred into a test tube and 5 ml of stock solution (0.375% thiobarbituric acid (w/v), 15% trichloroacetic acid (w/v) and 0.25 M HCl) was added. The tube was heated in boiling water for 15 min until a pink color appeared, cooled rapidly and centrifuged at 3000 rpm for 15 min to remove the flocculent precipitate. The absorbance of the clear solution was measure at 532 nm against a blank that contains all the reagents except for the sample. The MDA concentration was expressed as milligrams per kilogram sample and was calculated based on a calibration curve, which was built by using 1,1,3,3-tetramethoxypropane (TMP) as a standard precursor of malonaldehyde.

3. Results and Discussion

3.1. Extraction of phenolic compounds
Preliminary study on different variables (Figure 2) resulted that the highest polyphenol and flavonoid yields were obtained when the material was extracted with 60% ethanol as a solvent, at 60°C, for 60 minutes and with a 1/20 S/L ratio. The total phenolic content (TPC) and total flavonoid content (TFC) of onion peel obtained were 168.49 ± 0.02 mg GAE/g DW and 216.43 ± 0.06 mg QE/g DW, respectively. Those values were remarkably higher than other published experiments [15, 21, 28, 29]. Besides the onion sources, it can be observed from table 1 that the variation of TPC, TFC values among different studies has been attributed mainly to the extraction methods and conditions.
Figure 2. Effect of different extraction conditions on total phenol content (TPC) and total flavonoid content (TFC).

Table 1. TPC and TFC of onion peel obtained at different extracting conditions.

| Extracting solvent          | Temp. | Time  | S/L ratio | Mixing conditions | TPC<sup>a</sup> | TFC<sup>b</sup> | Reference |
|-----------------------------|-------|-------|-----------|-------------------|-----------------|----------------|-----------|
| EtOH:H<sub>2</sub>O 60:40 (v/v) | 60°C  | 60 mins | 1/20      | Magnetic stirring | 168.49         | 216.43        | Current study |
| EtOH:H<sub>2</sub>O:HCl 70:29.5:0.5 (v/v/v) | 35°C  | 90 mins | 1/20      | Shaking           | 52.7           | 43.1          | [28]      |
| Sub-critical water          | 165°C | 10 mins | -         | -                 | 25.0           | -             | [15]      |
| MeOH:H<sub>2</sub>O 80:20 (v/v) | 30°C  | 48 hs   | 1/5       | Magnetic stirring | 7.85           | 5.38          | [29]      |
| MeOH:Ace: H<sub>2</sub>O 7:7:6 (v/v/v) | 30°C  | 20 mins | 1/30      | Ultrasonicated    | 51.42          | 22.21         | [21]      |

<sup>a</sup> TPC (mg GAE/g DW), <sup>b</sup> TFC (mg QE/g DW)

As illustrated in table 1, compared to other solvent systems, ethanol-water was the most efficient to extract phenolic, flavonoid compounds from onion peels. Ethanol was chosen as a solvent for this study due to its safety, its versatility and its high ability to dissolve phenolic compounds rich in OH groups through hydrogen bonding. Phenolics in onion peels were reported to exist in two forms: aglycon and glycosides. Most phenolic glycosides, however, were more soluble in water. Therefore, different ethanol-water compositions gave different yields of TPC, TFC (Figure 2). From preliminary experiments, it was found that 60% ethanol gave the highest levels of total phenolic and flavonoid compounds.
Besides solvents, different extraction temperatures, times, solid/liquid ratios and methods of mixing were proved to affect significantly on TPC, TFC. It can be seen from Figure 2, the yields decreased noticeably at low temperature (40°C), short extraction time (30 minutes) and low solid/liquid ratio (1/10). Higher temperature increases the solubility of phenolic compounds, but too high temperature may lead to degradation of thermolabile compounds. Also, if the S/L ratio was too low, it would impede the stirring and so decrease the mass transfer rate.

J Pe’rez-Jime´nez et al [30] has evaluated the total phenolic contents of 100 richest dietary sources of polyphenols, which were various spices and dried herbs, cocoa products, some darkly colored berries, some seeds (flaxseed) and nuts (chestnut, hazelnut) and some vegetables, including olive and globe artichoke heads. The highest TPC was discovered in cloves (151.88 mg GAE/g DW) and dried peppermint (119.6 mg GAE/g DW). Onion waste, thus, can be concluded to be a valuable source of worthy phenolic compounds.

3.2. DPPH assays

Oxidative reactions are responsible for the deterioration of fats and oils which reduces nutritional quality of foods. Antioxidants help to prolong the shelf life of fatty foods through the ability to prevent the formation of free radicals, scavenge them or by accelerating their decomposition. The inhibition percentage of the extract in DPPH assays is given in Figure 3. DPPH radical scavenging activity of onion peel extract (IC\textsubscript{50} = 12.48± 0.45 µg/mL) was stronger than vitamin C (IC\textsubscript{50} = 18.87 ± 0.43 µg/mL) and comparable to that of butylated hydroxyl toluene (BHT, IC\textsubscript{50} = 10.65 ± 0.31 µg/mL), one of most common and powerful synthetic antioxidants used in food preservation. This result was correlated with the high content of polyphenols, especially flavonoids of the extract [31]. The same observation was reported in the work of Marija Lesjak et al [32], whereas IC\textsubscript{50} of BHT and Allium Cepa plant extract was about 10 and 30 µg/ml in DPPH assays, respectively. Onion skin was reported to constitute the highest concentration of flavonoid substances compared to the whole plant, it’s reasonable that the skin extract had a much lower IC\textsubscript{50} than of the whole plant. It should be mentioned that flavonoids have long been recognized as strong radical quenchers due to their ability to donate hydrogen atoms and terminate peroxyl radicals [33]. Flavonoids from onion peel extract were primarily composed of quercetin and quercetin glucosides [16]. Interestingly, DPPH scavenging capacity of quercetin (IC\textsubscript{50} = 1.5 µg/ml) was considerably higher than quercetin-3,4′-di-O-glucoside (IC\textsubscript{50} = 65 µg/ml) due to higher number of free hydroxyl groups [33]. Consequently, different ratios of quercetin and quercetin glucosides of the extract would affect its ability to extinguish DPPH radicals.

![Figure 3. DPPH scavenging activity (% inhibition vs concentration) of onion peel extract, vitamin C and BHT](image1)

![Figure 4. Quercetin structure](image2)
3.3. Tyrosinase inhibition assay

Tyrosinase is a copper-binding enzyme that plays a key role in the melanin biosynthesis in humans and animals [34]. The inhibition of tyrosinase is considered an effective method for the alleviation of skin hyperpigmentation and has recently attracted strong interest in lightening cosmetic products. Flavonoids with the ability to chelate metal ions due to their polyhydroxy phenolic structures have been reported as promising candidates for tyrosinase inhibition [35, 36]. The experiment showed that tyrosinase inhibition activity of the extract was minor. At the concentration of 1000 µg/mL, % inhibition was only 2.55% while that of Kojic acid was 99.97%. The extract’s low ability may be due to its flavonoid composition. Didem et al [35] have found that A and C ring of the flavonoid structure (figure 4), hydroxyl substituent at the 7th position, and hydroxyl substituents at para or para and meta position of ring B play a key role in competitive inhibition of the enzyme. Another research has observed that the methoxy and hydroxyl groups in the main flavanone skeleton play an important role in tyrosinase inhibition [37]. Conducting the assay on tyrosinase inhibition capacity of 20 flavonoids and related compounds, Isao Kubo et al found that the chelation mechanism seems to be specific to flavonols as long as the 3-hydroxyl group is free [38]. As mentioned above, the main flavonoids in onion peel were quercetin, quercetin-3,4’-O-diglucoside and quercetin-4’-O-mono-glucoside [7,8], it can be deduced that those glucosides are weak tyrosinase inhibitors due to lacking OH groups at 3, 4’ positions. Although quercetin was reported to be the most active flavanol, Chen Q-X et al [39] have demonstrated that the inhibition of tyrosinase by quercetin is a slow and reversible reaction. Quercetin displayed only 20% of the inhibitory strength of kojic acid toward diphenols activity of mushroom tyrosinase [40]. This result, therefore, was in agreement with previous reports and it could be concluded that despite being high source of flavonoids, onion skin extract is ineffective in tyrosinase inhibition.

3.4. α-glucosidase inhibition assay

α-glucosidase is the most important enzyme in carbohydrate digestion which hydrolyzes oligo- and disaccharides into glucose resulting in increasing glucose absorption and hyperglycemia. Alpha-glucosidase inhibitors play a role in delaying the decomposition and absorption of dietary carbohydrates by restricting the breakdown of oligosaccharides [41] Many pre-clinical studies reported antidiabetic effects of flavonol-rich extracts from different Allium spp. in vitro and in vivo [42, 43]. Ethanolic extract of Korean onion skin (6.04 g quercetin/100 g dried weight of onion skin) was reported to lower postprandial blood glucose response in diabetic rats and the effects are ascribed to the inhibition of carbohydrate digestive enzymes including α-amylase, α-glucosidase, and sucrose [44]

On alpha-glucosidase inhibition activity, the extract (IC₅₀=142.53 ± 0.16 µg/ml) exhibited about 14 times less potency than acarbose (IC₅₀ = 9.60 ± 0.11 µg/ml), an anti-diabetic drug used to treat diabetes mellitus type 2. Quercetin was declared to be more potent than or comparable with acarbose [42, 45, 46]. Many studies pointed out that hydroxylation at C3, C3’, C4’ is critical for the inhibitory activity of flavonoids [42, 46, 47]. The extract composed mainly of quercetin and quercetin-3,4’-O-diglucoside, quercetin-4’-O-monoglucoside, thus, possesses reduced capacity than quercetin.

This result was quite close with the work from Lee et al [48], of which methanol extract of onion skin inhibited yeast α-glucosidase with an IC₅₀ = 159 µg/mL. Also, their research found that one oral administration of the onion skin extract (500 mg/kg) could considerably lower the postprandial area under the glucose response curve to starch (1g/kg, p<0.05). Rats being fed with diets supplemented with onion skin extract displayed significantly reduced levels of plasma glucose, insulin, and blood glycated haemoglobin as compared with the control group.

3.5. Antibacterial assay
Food spoilage is the most prominent issue in food quality and safety. The main cause of food spoilage is invasion by microorganisms such as molds, yeast and bacteria. Bacterial contamination is more dangerous because very often food appears quite normal even though severely infected. The presence of highly dangerous toxins and bacterial spores is often not detected until after an outbreak of food poisoning [49]. Natural antimicrobial agents recently have gained much attention due to people’s increasing awareness of the negative impact of synthetic preservatives on health. Among natural products, phenolic, flavonoid compounds have been well documented to exhibit strong antibacterial capacity [50].

Disc diffusion test (Figure 5) illustrated that the extract solution in ethanol could inhibit both gram-positive (Bacillus subtilis, Staphylococcus aureus) and gram-negative (Escherichia coli, Salmonella, Pseudomonas aeruginosa) food-borne bacteria. The inhibition zone was in the range of 10.10 – 12.75 mm (Table 2). Control solutions containing only the solvent ethanol under identical experimental conditions showed no inhibition zone.

| Bacteria                         | Zone of inhibition (D, mm) | MIC(µg/mL) |
|----------------------------------|---------------------------|------------|
| Bacillus subtilis                | 11.03 ± 0.21              | 175        |
| Staphylococcus aureus            | 10.25 ± 0.29              | 200        |
| Escherichia coli                 | 11.08 ± 0.30              | 175        |
| Salmonella                       | 10.10 ± 0.26              | 200        |
| Pseudomonas aeruginosa           | 12.75 ± 0.21              | 150        |

The present study showed lower and better MICs than the results of Sagar et al [51]. In their research, extracts from Allium cepa skin of 15 Indian cultivars of different skin colors (dark red, red, pink and white) have been investigated. It was reported that the total flavonoid contents of onion skin extracts range from 1.31 to 168.77 mg QE/g DW. Hissar-3 cultivar (pink) displayed most effective against Bacillus subtilis and Salmonella (MIC = 0.45; 0.27 mg/ml respectively). Phursungi Local
The cultivar (pink) was most effective against *Staphylococcus aureus* (MIC = 0.54 mg/ml) and most cultivars displayed no or weak inhibition against *Pseudomonas aeruginosa*. The higher antimicrobial capacity of onion peel extract determined in this work may arise from different extract compositions due to the extraction method and higher flavonoid level. The results indicate that yellow onion skin is promising as an antimicrobial agent in food.

3.6. Evaluate the preservative effect of the extract on fish model

Lipid oxidation in meat and fish products is a major concern during processing and storage because it leads to rancid taste, off-flavor and so quality deterioration. Fish rich in polyunsaturated fatty acids (PUFA) are more susceptible to lipid peroxidation due to oxidation of numerous double bonds which restricts storage time and causes nutritionally losses.

Basa catfish, a nutritionally valuable fish with high content of protein and n-3 PUFA was chosen as a fish model to evaluate the extract’s potential in inhibiting lipid oxidation during different storage conditions. The lipid peroxidation process was monitored through two indicators: peroxide value (PV) and TBARS value. PV determines the amount of hydroperoxides, which are among the primary products and TBARS (Thiobarbituric reactive substances) measures malondialdehyde (MDA), a secondary product from lipid peroxidation of PUFA. Thiobarbituric acid reacts with MDA to form a pink TBA-MDA complex with characteristic absorption at 532 nm [52]. Two storage conditions were examined: cold 4°C for a 72h period and freezing (-18°C) for a 1 to 4 weeks period. Fish fillet samples coated with extract suspension (ES) of different concentrations (0.5-5%) were tested in accompany with blanks (untreated samples) and BHT (200ppm) treated samples for comparison.

**Preservative test at 4°C storage condition**

![Figure 6](image)

**Figure 6.** Plot of PV (left) and TBARS values (right) of fish sample versus storage times at 4°C.

As depicted in the **figure 6** under cold storage condition, PV and TBARS values of all samples increased gradually over time. However, the rising rate of extract-treated samples was lower compared to the blank and the more concentrated ES the more pronounced the effect. At concentrations 3% and 5%, PV and TBARS values of the extract-treated samples were almost coincident with that of the BHT-treated sample, which mean that the extract’s protective action was nearly equal to BHT.

Sensory observation showed that after 24h, blank samples and 0.5%, 1% extract-treated samples were smelly and the samples appeared to lose their firmness. Samples incorporated with 2%-5% ES were still in good conditions.

After 48h - 72h, signs of disintegration were observed on all samples. 1% ES treated samples presented a bad odor and the color became paler. Samples with 2-5% ES and with BHT were still seen as good appearance with a little smell but the resilience and firmness were diminished.
Preservative test at -18°C storage condition

Figure 7. Plot of PV (left) and TBARS values (right) of fish sample versus storage times at -18°C.

Under freezing condition, 3% ES exhibited nearly the same capacity as BHT in suppressing the lipid oxidation of the fish sample (figure 7). Compared with blank samples, PV and TBARS values of 3% ES extract treated samples were significantly lower. After four weeks, the appearance, odour of the fish samples was still in satisfactory state, the texture was just slightly softer than the fresh sample. The onion skin extract proved to effectively delay the lipid degradation and extend the self-life of fish meat during storage.

Onion skin as a source of antioxidant assisting food preservation has been demonstrated on many food models. Shim et al [53] has found that supplement of onion skin ethanol extract (0.2%) significantly improved the PV and TBARS values in raw ground pork meat after 16 days chilled storage. Yellow onion extract was also effective in inhibiting the oxidation of cooked salmon, TBARS values decrease 48.71% compared to the control at the end of 7 days storage at 4°C [21]. Incorporation of 1-2% onion peel powder into cooked sausages made from mechanically separated fish meat was reported to help enhance the sample sensory properties after 28 days at 5°C [54]. Moreover, ethanol onion skin extract also displayed to eliminate food microbial spoilage. Seasoned chicken breast meat treated with onion peel remarkably improved the microbial quality of the sample during aerobic storage at 4°C, 10°C and 20°C by reducing the initial counts of the microbial flora compared to control [55]. This study outcome was in consistent with other authors’ research to support the feasibility of onion peel skin as an alternative natural antioxidant agent to replace synthetic preservatives. However, more investigation should be done to assess the preservative action of the extract for practically longer storage time.

4. Conclusion
In summary, with many beneficial attributes: outstanding content of phenolics, flavonoids, potent antioxidant and antimicrobial capacity, strong α-glucosidase inhibitory activity and especially effective preservative action owning to synergistic interaction of all above properties, onion peel should be reconsidered as a valuable supplement source of biologically active phytochemicals to be exploited in food industry.

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