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Antioxidant Potential of Guava Leaf Extracts and Its Preservative Effects on the Shelf Life of the Grass Carp (*Ctenopharyngodon idella*) Fillets Under Refrigerated Condition

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ABSTRACT

The antioxidant activity of guava leaf extracts (GLE) was evaluated for their efficacy in enhancing the quality and extending the shelf life of grass carp (*Ctenopharyngodon idella*) fillets stored at a refrigerated temperature of 4 ± 1 °C for 16 days. The phytochemical content and antioxidant capacities of GLE were first determined, followed by periodic assessments of chemical, bacteriological, and sensory attributes in control and ethanolic GLE-treated fillets at concentrations of 1, 2, 3, and 4%. The results revealed that the absolute ethanol extracts of guava leaves contained the highest total phenolic content (170.32 mg GAE/g extract), total flavonoid content (51.98 mg QE/g extract), and antioxidant activity, significantly higher ($P< 0.05$) than 50% ethanol and water extracts. During the storage period, fish fillets treated with 3 and 4% GLE showed notably lower levels of pH, free fatty acids (FFA), thiobarbituric acid reactive substances (TBARS), and total volatile basic nitrogen (TVB-N) compared to the control and other treated groups. The highest aerobic plate count (APC) was reached on day 8 in the control group (7.24 log CFU/g), followed by the 1% (7.02 log CFU/g) and 2% GLE treatments (7.18 log CFU/g). In contrast, the 3 and 4% GLE treatments delayed microbial growth, reaching their highest APC values on day 16 (6.97 and 6.77 log CFU/g, respectively). Sensory evaluation showed that the 3 and 4% GLE-treated fillets maintained acceptable quality until day 12 of storage, whereas the control fillets were deemed unacceptable after just 4 days. Since no significant difference was found between the 3 and 4% treatments, the study suggests that 3% GLE is an effective, plant-based preservative for maintaining the quality and extending the shelf life of grass carp fillets during refrigeration.

INTRODUCTION

Grass carp (*Ctenopharyngodon idella*, Valenciennes, 1844) is extensively cultured and widely accepted in Bangladesh due to its white meat, delicious taste, rapid growth, ease of cultivation, efficient feed conversion, and rich nutritional content. Bangladesh's total fish production reached 4.915 million tonnes in 2022–2023, with grass carp contributing 85,843 tonnes **(DoF, 2023)**. Grass carp is typically marketed either fresh or in iced condition. Fish are considered highly perishable food items, prone to rapid spoilage and susceptible to microbiological and chemical deterioration **(Kundu *et al.*, 2024)**.

Various preservation methods, including the use of synthetic additives, are commonly employed to reduce quality loss and inhibit bacterial activity in fish. However, these methods are often ineffective in preventing overall quality degradation. Furthermore, improper use of chemical preservatives has been linked to an increased risk of cancer in humans **(Mei *et al.*, 2019)**. In contrast, natural extracts have demonstrated more effective preservative properties compared to synthetic additives **(Ahmed *et al.*, 2019)**. Consequently, scientists are increasingly interested in using natural preservatives containing bioactive compounds to maintain the quality and extend the shelf life of refrigerated fishery products.

Natural preservatives derived from medicinal plants, especially those rich in polyphenols, have exhibited antioxidant and various pharmacological properties **(Viji *et al.*, 2017)**. Plant-based preservatives are gaining global attention for their non-toxic nature. Guava (*Psidium guajava* L.), a popular fruit in Bangladesh, is rich in a variety of secondary metabolites—such as phenols, flavonoids, saponins, tannins, ferulic acid, caffeic acid, terpenoids, and phytosterols—that demonstrate strong antioxidant, antibacterial, and immunostimulant activities **(Kumar *et al.*, 2021)**. The flavonoids and polyphenols in guava help prevent the propagation of free radicals and delay oxidative rancidity **(Gowri & Manjunathan, 2020)**. Additionally, guava leaf extract (GLE) has shown potent antibacterial activity **(Melo *et al.*, 2020)**.

Multiple studies have confirmed the preservative potential of GLE in various fishery products, including mackerel **(Riyanto *et al.*, 2020)**, shrimp patties **(Yaqoob *et al.*, 2020),** dried anchovy **(Minh, 2021)**, and cobia fillets **(Tran *et al.*, 2021)**. Furthermore, GLE effectively inhibits lipid oxidation, suppresses bacterial growth, and extends the shelf life of snakehead fillets **(Huynh *et al.*, 2021)**. However, no studies have yet investigated the application of GLE in preserving grass carp fillets.

Therefore, this study aims to evaluate the antioxidant activities of GLE extracted using various solvents and assess its potential in preserving grass carp fillets under refrigerated conditions at 4 ± 1°C.

MATERIALS AND METHODS

Materials and chemicals

Guava (*P. guajava* L.) leaves were obtained from local cultivators in Gazipur, Bangladesh. The freshly harvested leaves were sun-dried at ambient temperature (26–28°C) for one week. Then, the leaves were segmented into small cuts and blended into a fine powder. The ground leaves were wrapped in plastic bags and preserved at –25 °C until use. Eight live grass carp (*C. idella*) (average weight 2830 ± 250 g) were procured from a fish market in Joydebpur, Bangladesh, and killed by immersion in ice-cold water. Whole *C. idella* were immediately iced (prepared from freshwater) in a protected ice box, maintaining an ice-to-fish ratio of approximately 2:1. The chilled fish were transported to the Fishery Product Development Laboratory in the Faculty of Fisheries at Gazipur Agricultural University (GAU), Bangladesh. All chemicals and reagents used were of HPLC or analytical grade.

Preparation of GLE

The guava leaf powder was extracted using distilled water (0E), 50% ethanol (purity >99.8%; 50E), and absolute ethanol (100E). Water extracts were prepared following the method of **Kandil *et al.* (1994)**, with minor modifications. Briefly, 100g of powdered guava leaf was boiled in 1.5L of distilled water for 15 minutes, then filtered through filter paper. The extract was concentrated using a rotary evaporator (Bibby Scientific Ltd., Stuart RE300/MS, Staffordshire, UK) and designated as the water extract (0E). For 50% ethanol and absolute ethanol extractions, 10g of guava leaf powder was soaked in 100mL of a water-ethanol mixture (1:1) and in absolute ethanol, respectively, for 72 hours at ambient temperature (26– 28°C) with periodic gentle shaking (**Afrin *et al.*, 2023**). The extract was filtered, and the residue re-dissolved in the same solvent. The resulting solution was concentrated using a rotary evaporator. The dried extracts were weighed to determine yield and stored at 4°C until analysis. Standard solutions were prepared by dissolving 1000μg of extract in 1.0mL of methanol.

Phytochemical content and antioxidant assays

The total phenolic content (TPC) of GLE was determined according to the method of **Chakma *et al.* (2023)** and expressed as mg GAE/g of extract. Total flavonoid content was also measured following **Chakma *et al.* (2023)** and reported as mg QE/g of extract. The DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid]) radical scavenging activities were evaluated as per **Chakma *et al.* (2023)**. EC_{50} values represent the concentration of extract required to scavenge 50% of DPPH or ABTS radicals. The CUPRAC assay was performed following **Sabudak *et al.* (2013)**, where $ECA_{0.50}$ indicates the extract concentration producing an absorbance of 0.50 ($A_{0.50}$).

Fish fillets preparation

Fish were filleted using sterilized sharp knives on sanitized cutting boards. The head and bones were removed to obtain two fillets from each fish, which were then sectioned into smaller pieces (average weight 49.2 ± 7.58 g). Only the anterior-dorsal muscle portions were used in the experiment.

Coating application and storage

Based on antioxidant activity results, ethanol extract was selected to determine the optimal GLE concentration for extending fish fillet shelf life under refrigerated storage. GLE was diluted in distilled water at various concentrations. Fillets were randomly divided into five treatment groups: control (0% GLE), 1% GLE, 2% GLE, 3% GLE, and 4% GLE, with three replicates each. Fillets were soaked in their respective solutions for 10 minutes at 4°C, then placed on plastic nets for 5 minutes to form edible coatings. Each fillet was sealed in a polyethylene zipper pouch and stored at 4 ± 1°C for 16 days. Chemical, bacteriological, and sensory analyses were performed on day 0 (within 2 hours of treatment) and every four days until visible spoilage.

Chemical analyses

Chemical properties of the fillets were analyzed following the **AOAC (2006)** method. pH was measured directly using a pH meter. Free fatty acids (FFA) were determined based on **Kirk and Sawayer (1991)** and expressed as percent oleic acid equivalents. TBARS were measured using the **Buege and Aust (1978)** method and expressed as mg MDA/kg of fish flesh. Total volatile basic nitrogen (TVB-N) was evaluated as per **AOAC (2006)** and reported in mg N/100 g of tissue.

Bacteriological analysis

Bacteriological properties were analyzed following **Afrin *et al.* (2021)**. Aerobic plate count (APC) and psychrophilic bacterial count (PBC) were assessed using the pour plate technique with plate count agar. APC plates were incubated for 24 hours at 37°C, and PBC plates for 10 days at 7°C. Results were reported as log CFU/g of fish tissue.

Sensory evaluation

Sensory quality of raw fish fillets was evaluated using a nine-point hedonic scale **(Peryam & Pilgrim, 1957)**. The panel consisted of twelve trained evaluators aged 21–33 years from the Fishery Product Development Laboratory at GAU. Scores ranged from 9 (like extremely) to 1 (dislike extremely). Fillets scoring above 5 were considered acceptable.

Statistical analyses

One-way ANOVA was used for antioxidant activity data, and two-way ANOVA was applied for fish fillet quality parameters. The study followed a completely randomized design with triplicate measurements. Duncan's multiple range test was employed to detect significant differences at $P < 0.05$. Data analysis was performed using Statistics 10 (Tallahassee, FL, USA).

RESULTS AND DISCUSSION

Yield and phytochemical content of GLE

The extract yield of GLE varied from 8.9 to 26.7% (Table 1). Significantly (P<0.05) higher yield was found in 100E, followed by 50E and 0E. Comparable result was reported by **Vieito *et al.* (2018)**, who observed a comparatively higher yield in the ethanolic extract of pine bark (*Pinus pinaster* sub sp. Atlantica), followed by aqueous ethanol and water extract. In another study, ethanolic extract of pear peel produced the highest yield followed by 80% ethanolic and aqueous extracts **(Patricia & Syaputri, 2021)**. The TPC of GLE varied from 111.04 to 170.32mg GAE/g (Table 2). The 100E extract presented the maximal TPC (170.32 mg GAE/g), followed by 50E (150.13mg GAE/g) and 0E (111.04mg GAE/g) (P< 0.05), indicating a decline in TPC with increasing solvent polarity. Likewise, the maximal TFC was recorded in 100E (51.98 mg QE/g extract), while 0E showed the minimal value (25.23mg QE/g) (Table 2). However, **Khedr *et al.* (2021)** found comparatively higher amounts of TPC (386.5mg GAE/g) and TFC (265.9 mg QE/g) in ethanolic extracts of guava leaf. **Gudise *et al.* (2019)** found that ethanol extracts of *Argyreia pierreana* leaf exhibited higher amounts of TPC (130.49mg GAE/g) and TFC (44.48mg QE/g) than aqueous extracts. Similarly, relatively higher amounts of TPC and TFC were found in the ethanolic extract than in hydroethanolic and water extracts of *Allium sativum controversum* **(Madani *et al.*, 2023)**.

Table 1. Yield and phytochemical content of guava leaf extracts[1]

Extract	Yield (g/100g of dry weight)	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
0E	8.9 ± 0.78^{c}	111.04 ± 3.03^{c}	25.23 ± 0.65^{c}
50E	22.8 ± 2.16^{b}	150.13 ± 5.52^{b}	47.43 ± 1.54^{b}
100E	26.7 ± 1.47^{a}	170.32 ± 3.87^{a}	51.98 ± 2.37^{a}

[1]Findings are interpreted as mean ± SD (n = 3). Superscript letters within a column denote variations (P < 0.05) of means.
Abbreviations: 0E = water extract; 50E = 50% ethanol extract; 100E = 100% ethanol extract.

Antioxidant activity of GLE

The antioxidant capacity of GLE in various solvents is depicted in Table (2). The lower EC_{50} value means a greater antioxidant capacity. The EC_{50} value of the DPPH radical scavenging activity of GLE fluctuated from 0.10 to 0.44mg/ mL. The maximal EC_{50} value was detected in 0E (0.44mg/ mL), followed by 50E (0.19mg/ mL), and 100E (0.10mg/ mL). Likewise, the maximal ABTS value (EC_{50}) was found in 0E (2.56mg/ mL), while the lowest EC_{50} value (0.83mg/ mL) was recorded in 100E. Moreover, the highest CUPRAC value ($EC_{A0.50}$) was detected in 0E (57.41µg/ mL), followed by 50E

(38.14µg/ mL), and 100E (5.52µg/ mL). These results suggest that ethanolic extracts of guava leaf exhibited a higher antioxidant capacity; this could be linked to the presence of a greater quantity of phenolic compounds. **Madani *et al.* (2023)** indicated that ethanol extract of *Allium sativum controversum* demonstrated the lowest DPPH (IC_{50}) value (8.29 mg/mL) when compared to hydroethanolic (10.09mg/ mL) and aqueous (11.85mg/ mL) extracts.

Table 2. Antioxidant activities of guava leaf extracts[1]

Extract	DPPH radical scavenging activity (EC_{50} [mg/mL])[†]	ABTS radical scavenging activity (EC_{50} [mg/mL])	Cupric ion reducing antioxidant capacity ($EC_{A0.50}$ [µg/mL])[‡]
0E	0.44 ± 0.01^{a}	2.56 ± 0.53^{a}	57.41 ± 6.07^{a}
50E	0.19 ± 0.003^{b}	1.09 ± 0.12^{b}	38.14 ± 6.64^{b}
100E	0.10 ± 0.01^{c}	0.83 ± 0.06^{c}	5.52 ± 1.43^{c}

[1]Findings are interpreted as mean ± SD (n = 3). Superscript letters within a column designate differences (P < 0.05) of means. [†]EC_{50} denotes the half-maximal effective concentration. [‡]$EC_{A0.50}$ means the effective concentration of absorbance of 0.50.
Abbreviations: 0E = Aqueous extract; 50E = 50% ethanol extract; 100E = 100% ethanol extract.

Proximate composition

The proximate composition of grass carp fillets was found to be 75.6% moisture, 18.21% crude protein, 4.26% crude fat, and 0.98% ash on a fresh matter. Numerous researchers have investigated the proximate composition of grass carp fillets, and the results from these studies vary from the present findings **(Pyz-Lukasik & Paszkiewicz, 2018; Matos *et al.*, 2019)**. Alterations in the proximate composition of fish flesh may fluctuate due to factors like diet, fish size, habitat, time of capture, feeding habits, gender, breeding cycle, and environmental influences **(Rasul *et al.*, 2021; Dulal *et al.*, 2023)**. Since grass carp are farmed fish, their chemical composition, particularly the amount of protein and lipids, may fluctuate based on the feed provided and the water quality in the farming environment.

Changes in pH values

The variations in pH of fish fillets throughout refrigerated conditions are illustrated in Fig. (1). The primary pH of both untreated and GLE-treated fillets varied from 6.30 to 6.35, indicating that the fillets were fresh before storage **(Afrin *et al.*, 2021)**. Within the first 4 days of storage, the pH values of both untreated and GLE-treated fillets were initially reduced. This early decline in pH is likely due to the accretion of lactic acid after glycogen breakdown in the fish tissue **(Cai *et al.*, 2014)**. After 4 days of refrigerated storage, the pH values significantly increased (P< 0.05), reaching 7.18, 7.03, 7.12, 7.15,

and 7.04 for control, 1%, 2%, 3%, and 4% GLE-treated fillets at 8th, 8th, 12th,16th and 16th days, respectively. An increase in pH was notably slower in GLE-treated fillets in comparison with the control that could be due to the antibacterial properties of phenolics from guava leaves, which helped to minimize the development of basic compounds **(Teshome *et al.*, 2022)**. Comparable finding was also demonstrated by **Rajasekar *et al.* (2020)**, who noted a gradual increase in pH in turmeric and seaweed-treated finfish (*Sillago sihama*) during 14 days of refrigerated storage. The successive rise in pH values is probably linked to the creation of nitrogen-bearing substances like ammonia and trimethylamine, resulting from bacterial activity or endogenic enzymatic processes **(Afrin *et al.*, 2021)**.

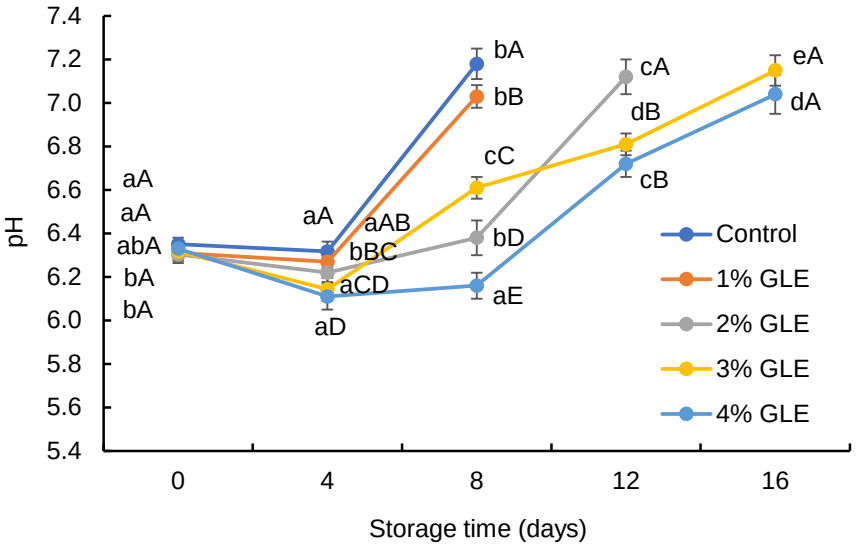

Fig. 1. Changes in pH of grass carp fillets under refrigerated condition. The data denote means ± SD (n=3). [a-e] Different lowercase letters of each line designate variations (P<0.05) of means between the preservation periods. [A-E] Different uppercase letters designate variations (P<0.05) among the samples on the same analysis day.

Changes in FFA

The FFA serves as a reliable indicator for measuring lipid ruin in fish under storage condition. The initial FFA content of all fish fillets ranged from 0.52 to 0.54% oleic acid (Fig. 2). Throughout the refrigerated storage, the FFA content of grass carp fillets were augmented significantly (P< 0.05), indicating that lipids in grass carp were continuously hydrolyzed **(Cao *et al.*, 2019)**. The maximal FFA values recorded were 2.44%, 2.14%, 2.25%, 2.53 %, and 2.25% oleic acid for control, 1%, 2%, 3%, and 4% GLE-treated fillets at the 8th, 8th, 12th, 16th, and 16th days of preservation, respectively, with all values exceeding the maximum acceptable limit of 1.5% oleic acid **(Pal *et al.*, 2017)**. During the entire storage duration, the development of FFA was fairly slower in GLE-treated fillets than the control fillets, suggesting that the phenolic molecules present in GLE may

significantly influence the reduction of FFA formation. **Minh (2021)** reported similar findings, indicating that GLE significantly reduced the FFA formation in dried Anchovy (*Stolephorus* sp.) during storage. Moreover, the lowest FFA content was found in 4% GLE-treated fillets as compared to 3% GLE-treated fillets after 16 days of storage, though the result shows non-significant variations ($P > 0.05$). **Mehdizadeh *et al.* (2019)** also found that rainbow trout fillets dipped into 6% *Salvia officinalis* extracts deferred the increasing rate of FFA content than the control samples at 4°C. **Pal *et al.* (2017)** also stated comparatively lower FFA content in green tea extract (0.61% oleic acid) and reuse tea leave extracts (0.64% oleic acid) treated samples than the control (2.02% oleic acid) samples kept at 4±1°C for 15 days. **Balikci *et al.* (2022)** also observed that thyme, rosemary, and basil extract effectively retarded FFA formation in the vacuumed-packed mackerel balls during the refrigerated condition.

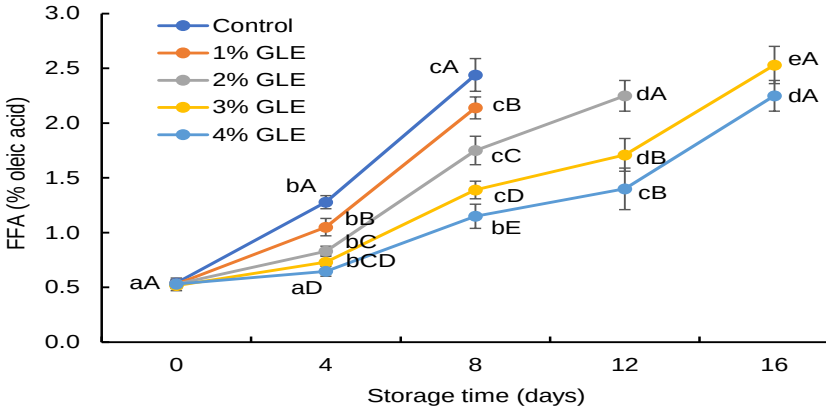

Fig. 2. Changes in free fatty acid (FFA) values of grass carp fillets under refrigerated condition. The data denote means ± SD (n=3). [a-e] Different lowercase letters of each line designate variations (P<0.05) of means between the preservation periods. [A-E] Different uppercase letters designate variations (P<0.05) among the samples on the same analysis day.

Changes in thiobarbituric acid reactive substances (TBARS)

The TBARS of all fillets at zero-day ranged between 0.31 mg MDA/kg and 0.32 mg MDA/kg of fish flesh (Fig. 3). The TBARS of both untreated and GLE-treated fillets showed a significant ($P < 0.05$) increase at different degrees during storage period. The highest TBARS were 0.77, 0.68, 0.82, 0.83, and 0.76 mg MDA/kg fish muscle for control, 1%, 2%, 3%, and 4% GLE-treated fillets at the 8th, 8th, 12th 16th, and 16th days of preservation, respectively, and the values remained within satisfactory limits (1-2 mg MDA/kg flesh) **(Connell, 1990)**. The TBARS of GLE-treated fillets were reduced (P< 0.05) and the augmentation rate was relatively slower compared to the control. This effect could be due to the occurrence of phenolic compounds in GLE that retard the production of secondary lipid oxidized compounds in the fillets **(Melo *et al.*, 2020)**. In the present

study, the fillets treated with 4.0% GLE exhibited significantly the lowest TBARS, and no notable difference (P> 0.05) was detected between the 3 and 4% GLE-treated fillets. Our findings are aligned with the observation of **Huynh *et al.* (2021)**, who demonstrated that *P. guajava* leaf extract effectively decreased the TBARS formation in the snakehead fillets during icing. Moreover, 15% ginger extract significantly reduced the TBARS value in tilapia fillets in comparison with control throughout the refrigerated storage **(Islam *et al.*, 2022)**.

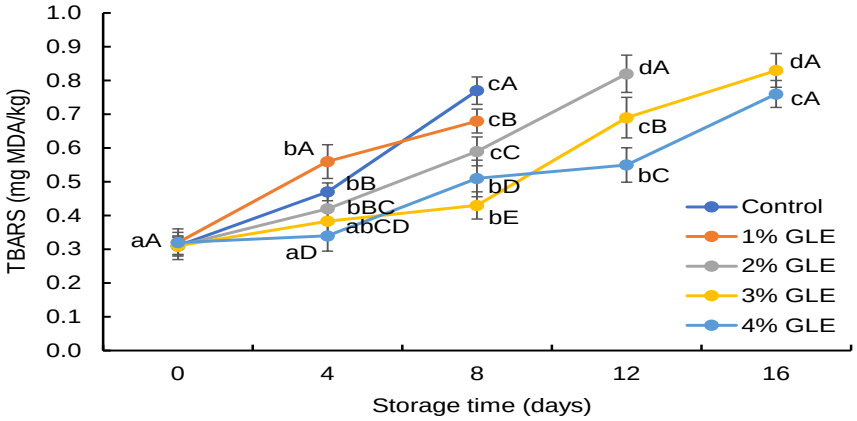

Fig. 3. Changes in thiobarbituric acid reactive substances (TBARS) values of grass carp fillets under refrigerated conditions. The data denote means ± SD (n=3). [a-d] Different lowercase letters of each line designate variations (P<0.05) of means between the preservation periods. [A-E] Different uppercase letters designate variations (P<0.05) among the samples on the same analysis day.

Changes in TVB-N

The TVB-N value of grass carp fillets on day 0 ranged from 7.06 to 7.16 mg N/100 g (Fig. 4). At the end of the storage period, TVB-N levels reached 33.70, 30.32, 32.10, 33.94, and 31.02mg N/100 g for the control, 1, 2, 3, and 4% GLE-treated fillets on days 8, 8, 12, 16, and 16, respectively. These values exceeded the maximum acceptable limit of 30mg N/100 g **(Dulal *et al.*, 2023)**. In this study, the relatively lower TVB-N values observed in GLE-treated samples may be attributed to reduced bacterial growth, suppression of bacterial activity linked to the oxidative degradation of non-protein nitrogenous (NPN) compounds, or a combination of both **(Ojagh *et al.*, 2010)**.

Consistent with these findings, **Tran *et al.* (2021)** reported that GLE effectively reduced TVB-N accumulation in cobia (*Rachycentron canadum*) fillets during 15 days of refrigerated storage. Likewise, refrigerated common carp fillets treated with 1.5% grape seed extract also exhibited significantly lower TVB-N values compared to the control **(Albashr *et al.*, 2021)**. However, in the current study, no significant difference (P > 0.05) was observed between the 3% and 4% GLE treatments.

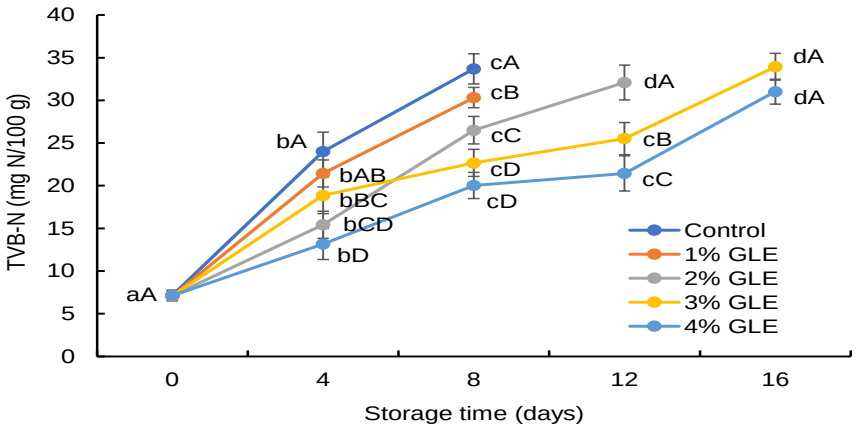

Fig. 4. Changes in total volatile basic-nitrogen (TVB-N) values of grass carp fillets under refrigerated condition. The data denote means ± SD (n=3). [a-d] Different lowercase letters of each line designate variations (P<0.05) of means between the preservation periods. [A-D] Different uppercase letters designate variations (P<0.05) among the samples on the same analysis day.

Bacteriological changes

Changes in APC

The APC of grass carp fillets at zero-day varied between 3.43 and 3.55 log CFU/g (Fig. 5a). Both GLE-treated and control fillets exhibited a significant rise (P< 0.05) in APC as the preservation period progressed. The highest APC for control, 1, and 2% GLE-treated fillets were 7.24, 7.02, and 7.18 log CFU/g found at the 8th, 8th, and 12th days of the preservation time, respectively, which surpassed allowable range (7 Log CFU/g tissue) for fresh fish **(ICMSF, 1986)**. However, 3% (6.97 log CFU/g), and 4% (6.77 log CFU/g) GLE-treated fish fillets were still within the allowable limit up to 16 days, and no significant (P> 0.05) deviation was found between them. The results suggest that polyphenolic components in the GLE effectively reduced bacterial growth in the GLE-treated fillets **(Das & Goswami, 2019)**. It has been reported that *Psidium guajava* and *Camellia sinensis* extracts effectively reduced the APC in the snakehead fillets up to 12 days of chilled storage **(Huynh *et al.*, 2021)**. In addition, **Olatunde *et al.* (2021)** observed that ethanolic GLE significantly inhibited bacterial growth in the Pacific white shrimp during refrigerated storage. Additionally, 0.2% of tea polyphenols significantly reduced the APC in the grass carp fillets under refrigerated conditions **(Pan *et al.*, 2021)**.

Changes in PBC

The proliferation of psychrophilic bacteria is the primary cause of spoilage in fish and seafood during refrigerated storage **(Olatunde & Benjakul, 2018)**. The primary PBC in grass carp fillets varied from 2.68 to 2.73 log CFU/g of fish flesh (Fig. 5b). The PBC was increased gradually during preservation, and the values surpassed the allowable range of 7 log CFU/g on the 8th, 8th, 12th, 16^{th,} and 16th days for the control, 1, 2, 3, and

4% GLE-treated fillets, respectively. The PBC appeared to be lower in GLE-treated fillets in comparison with control fillets, likely due to the existence of tannin and flavonoids in GLE **(Naili *et al.*, 2020)**. **Khidhir (2015)** found that 4% olive leaf extract (OLE) significantly reduced PBC in *Caprinus caprio* fillets as compared to 2% OLE treated and control fillets after 7 days of chilled storage. **Yazgan *et al.* (2020)** found that ethanolic propolis extract (0.8%) effectively inhibited the proliferation of psychrophilic bacteria in sardine flesh during refrigerated conditions. Moreover, peppermint combined with chitosan effectively reduced TVC and PBC in *Cyprinus carpio* fillets under refrigerated condition **(Morachis-Valdez *et al.*, 2021)**.

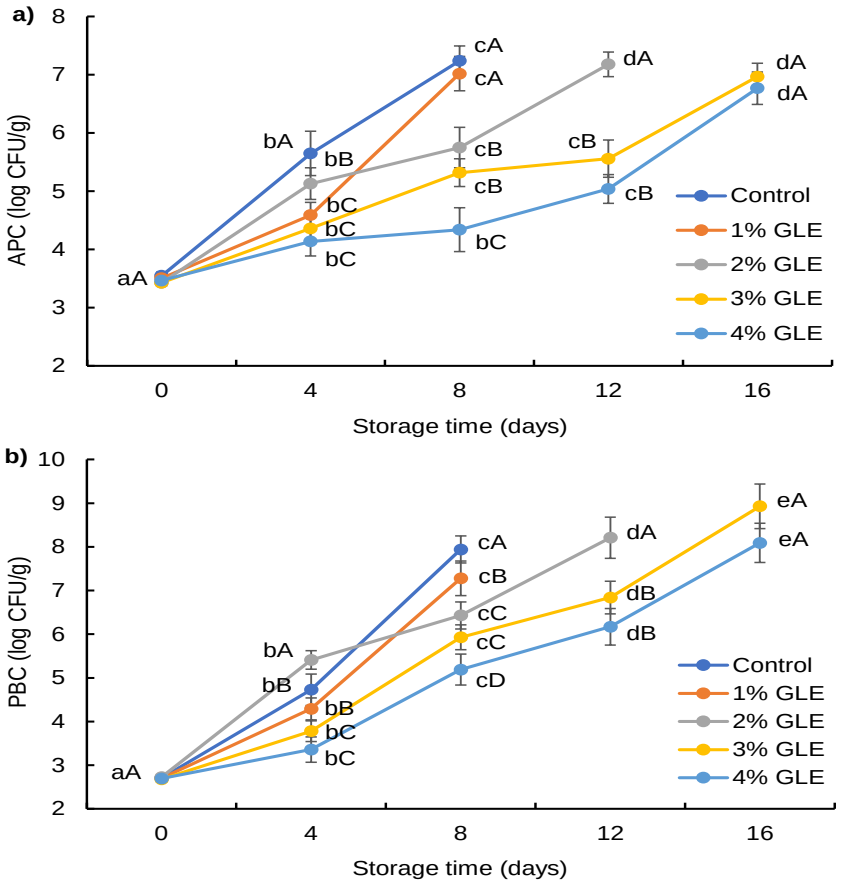

Fig. 5. Changes in (a) aerobic plate count (APC) and (b) total psychrophilic count (TPC) of grass carp fillets under refrigerated conditions. The data denote means ± SD (n=3). $^{a\text{-}e}$ Different lowercase letters of each line designate variations (P<0.05) of means between the preservation periods. $^{A\text{-}E}$ Different uppercase letters designate variations (P<0.05) among the samples on the same analysis day.

Sensory evaluation

Sensory properties of food products are known as key factors in consumer acceptance. Sensory assessment showed that the control, 1, 2, 3, and 4% GLE-treated

fillets became undesirable at the 8th, 8th, 12th, 16th, and 16th days, respectively (Table 3). The sensory qualities were found acceptable up to 12 days of storage in 3% and 4% GLE-treated fillets. It was also observed that the presence of GLE improved the organoleptic quality of the GLE-treated fillets, which could be attributed to the occurrence of polyphenols in guava leaf, which helps to retain the color, decrease fishy odor, and preserve the quality of grass carp fillets **(Pal *et al.*, 2017; Yaqoob *et al.*, 2020; Shao *et al.*, 2022)**. Color was formed in the GLE-treated fillets during dipping, although no negative effect was found in the sensory attributes. However, muscle elasticity, color, and odor gradually decreased with the increasing storage time, which could be associated with the changes in the fish muscle constituents by lipid and protein oxidation, enzymatic, and microbial activity **(Tran *et al.*, 2021)**.

Table 3. Sensory attributes of grass carp fillets under refrigerated condition[1]

Storage time (days)	Control	1.0% GLE	2.0% GLE	3.0% GLE	4.0% GLE
Color					
0	8.96 ± 0.05aA	8.94 ± 0.04aA	8.91 ± 0.08aA	8.89 ± 0.07aA	8.86 ± 0.13aA
4	7.76 ± 0.11bB	7.91 ± 0.15bB	8.64 ± 0.23aA	8.76 ± 0.08aA	8.81 ± 0.18aA
8	4.10 ± 0.09cC	4.22 ± 0.26cC	6.61 ± 0.24bB	7.32 ± 0.21bA	7.46 ± 0.34bA
12			3.98 ± 0.40cB	6.39 ± 0.22cA	6.57 ± 0.17cA
16				3.89 ± 0.26dA	3.94 ± 0.18dA
Odor					
0	8.98 ± 0.01aA	8.89 ± 0.07aA	8.86 ± 0.09aA	8.78 ± 0.12aA	8.76 ± 0.08aA
4	6.77 ± 0.17bC	7.48 ± 0.15bB	8.37 ± 0.18bA	8.49 ± 0.13aA	8.51 ± 0.09aA
8	3.81 ± 0.39cC	4.22 ± 0.12cC	6.71 ± 0.48cB	7.78 ± 0.16bA	7.85 ± 0.16bA
12			3.95 ± 0.04dB	6.01 ± 0.41cA	6.06 ± 0.37cA
16				3.67 ± 0.17dA	3.77 ± 0.20dA
Texture					
0	8.97 ± 0.01aA	8.96 ± 0.03aA	8.93 ± 0.02aA	8.91 ± 0.05aA	8.87 ± 0.08aA
4	6.89 ± 0.27bC	7.17 ± 0.16bC	8.13 ± 0.17bB	8.25 ± 0.14bB	8.31 ± 0.19aA
8	3.87 ± 0.21cC	4.15 ± 0.33cC	6.75 ± 0.12cB	7.73 ± 0.38cA	7.86 ± 0.13bA
12			3.98 ± 0.17dB	6.27 ± 0.15dA	6.48 ± 0.27cA
16				3.89 ± 0.18eA	4.12 ± 0.27dA
Overall acceptability					
0	8.97 ± 0.02aA	8.94 ± 0.03aA	8.96 ± 0.02aA	8.91 ± 0.06aA	8.95 ± 0.03aA
4	7.43 ± 0.13bC	7.67 ± 0.15bB	8.34 ± 0.18bA	8.42 ± 0.14bA	8.57 ± 0.15aA
8	3.91 ± 0.16cC	4.10 ± 0.13cC	6.30 ± 0.20cB	7.65 ± 0.42cA	7.76 ± 0.42bA
12			3.97 ± 0.11dB	6.15 ± 0.19dA	6.27 ± 0.12cA
16				3.79 ± 0.21eA	3.83 ± 0.32dA

[1]Sensory scores are stated as mean ± SD (n=12); Small letter denotes variations (P<0.05) between storage times within the treatments, while the capital letter denotes variations (P<0.05) among treatments on same analysis day. A minimum sensory value of 5.0 denotes threshold for fresh fish. Abbreviation: GLE, Guava leaf extracts.

CONCLUSION

The application of natural additives like guava leaf extract (GLE) to extend the shelf life of fish fillets has gained importance due to growing consumer awareness and demand for healthier food alternatives. In this study, the ethanolic extract of guava leaf exhibited the highest total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity, followed by the 50% aqueous ethanol and aqueous extracts. Throughout the storage period, GLE-treated fillets showed comparatively lower levels of pH, TBARS, FFA, and TVB-N. Additionally, the aerobic plate count (APC), psychrophilic bacterial count (PBC), and sensory attributes of fillets treated with 3 and 4% GLE remained within acceptable limits up to the 12th day of storage.

The ethanolic GLE, rich in secondary metabolites, effectively inhibited the formation of FFAs, TVB-N, and other lipid oxidation byproducts, while also suppressing bacterial growth. These effects contributed to an extended shelf life and better preservation of fillet quality. Based on chemical, bacteriological, and sensory analyses, and considering statistical significance, 3% GLE is recommended for maintaining the quality and extending the shelf life of refrigerated grass carp fillets. Therefore, ethanolic GLE shows strong potential as a natural additive for fish preservation.

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