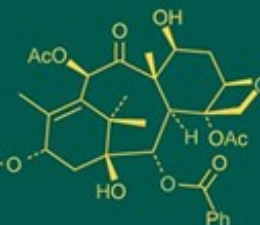
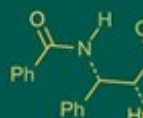
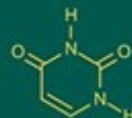


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Effect of mint leaf extract on the shelf life and quality attributes of Indian mackerel, *Rastrelliger kanagurta* (Cuvier, 1816) steaks stored at chilled temperature

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ABSTRACT

The present study investigated the effect of mint leaf extract (MLE) on the quality and shelf life of Indian mackerel (*Rastrelliger kanagurta*) steaks stored under chilled conditions. The primary objective was to assess the phenolic content of the mint extracts and evaluate their impact on the shelf life of the fish steaks. Indian mackerel steaks were dipped in a mint leaf extract (concentrations of 0.4%, 0.5%, and 0.6%), vacuum-sealed in HDPE bags, and stored at refrigeration temperature. The quality was evaluated based on biochemical, microbiological, and sensory parameters. Biochemical indices such as total volatile base nitrogen (TVB-N), trimethylamine nitrogen (TMA-N), and peroxide value (PV) showed significant ($p < 0.05$) reductions in the extract-treated fish compared to the control group. The total plate count (TPC) was notably higher in the control samples compared to those treated with the mint leaf extract. Sensory evaluation revealed that the shelf life of the treated fish steaks was extended up to 9 days, whereas, the control samples maintained quality for only 6 days.

Keywords: Mint leaf extract, Indian mackerel, Shelf life

1. Introduction

In recent years, a rising need for wholesome, sustainable, and high-protein food sources has led to a major increase in interest in fish food items. Fish, especially from sustainable aquaculture, has become a popular choice as the world's population grows and people become more conscious of the need for good meals. This is because fish is high in protein, omega-3 fatty acids, and other vital elements. The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations (UN) recommend regular fish consumption of 1-2 meals per week to provide a comparable amount of 200-500 mg of n-3 PUFAs, particularly eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) (FAO/WHO, 2011). The current situation demonstrates a trend toward functional fish products that offer additional health advantages in addition to satisfying nutritional needs. The industry is also being driven by value-added items like ready-to-eat fish dinners and advancements in preservation methods such as using natural extracts to prolong shelf life. Concerns around sustainability, food safety, and environmentally friendly methods are also impacting the creation of fish food products that are beneficial to consumers' health.

Indian mackerel (*Rastrelliger kanagurta*) is a commercially important species harvested in large quantities along the Indian coast (Ganga, 2010) [1]. The total fish production of India in 2024-25 was 197.75 lakh tonnes, out of which 46.15 and 151.60 lakh tonnes were marine and inland fish production, respectively (Handbook of Fisheries Statistics, 2025). The total production of Indian mackerel in India along the east coast was 0.77 lakh tonnes and along the west coast 1.42 lakh tonnes, respectively (Handbook of Fisheries Statistics, 2025).

Plants in the Lamiaceae family are abundant in phenolic compounds, which have been found to have strong antioxidant properties (Ozgen *et al.*, 2006; Tepe *et al.*, 2007) [2, 3]. Mints are aromatic herbs belonging to the Lamiaceae family, namely the *Mentha* genus. *Mentha* has about 30 species worldwide, with the majority of them found in temperate and tropical/subtropical climates (Taneja *et al.*, 2012) [4]. *Mentha* consists of essential oil, menthol, and other chemical components. *Mentha* is employed in a range of industries including food, perfumes, and pharmaceuticals (Taneja *et al.*, 2012) [4]. Mint extracts have been shown to have antioxidant activity comparable to synthetic antioxidants (Kanatt *et al.*, 2008) [5]. Rich in antioxidants like phenolic compounds, ascorbic acid, and carotenoids, mint helps prevent oxidation (Park *et al.*, 2019) [6]. Mint extract is recognized as a natural preservative for fish and fishery products, effectively reducing microbial growth, delaying chemical deterioration, and enhancing sensory properties, thus extending shelf life (Raisi *et al.* 2015) [7]. Mint leaf has the potential to be utilized as a natural preservative in fish and fishery products (Viji *et al.*, 2015) [8]. In the present study, concentrations have been used for mint treatment, based on the re

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search carried out by (Viji *et al.*, 2015) [8], in that paper the authors stated that a fish treated with 0.5% concentration of mint extract showed the best biochemical quality and extended shelf life (16–17 days), compared to 11–13 days for the control.

Seafood is an essential component in human diets, it is vital to maintain the quality of seafood and extend shelf life. Natural preservatives prolong the shelf life of seafood but also secure human health and contribute significantly to the reduction of both food loss and poor nutrition. Mint leaf extract increased the antioxidant and antimicrobial properties of Indian mackerel. The successful use of mint leaf extract can be used to enhance the physiochemical and sensory properties of Indian mackerel.

The current study will investigate the impact of extracts made from mint leaves as natural preservatives for enhancing the quality and shelf life of Indian mackerel steaks at chilled temperatures, taking into consideration the importance of fish quality during chilled storage.

2. Materials and Method

2.1 Raw material collection

Indian mackerel (*Rastrelliger kanagurta*) was procured from the Mirkarwada, Ratnagiri by maintaining a chilled condition with 1:1 (w/w), ice: fish in insulated boxes. Fishes used for the study had total length and total weight of 21 to 24 cm and 150 to 200 gm, respectively. Following dressing and washing, the fish was cut into 2.0 to 2.5 cm size steaks, yielding $66.33 \pm 0.81\%$ from the whole mackerel fish. The mint (*Mentha arvensis*) leaves were sourced from the local vegetable market of Ratnagiri. All the chemicals and glassware used were of reagent grade.

2.2 Preparation of ethanol extract of mint (*Mentha arvensis*) leaf

Fresh mint leaves were sourced from the local vegetable market of Ratnagiri, cleaned and washed thoroughly under continuous running water. Ethanol extract of mint leaves was prepared by modifying the protocol described by (Biswas *et al.*, 2012) [9]. The excess water was

drained and the leaves were dried in a cabinet dryer at $50 \pm 2^\circ\text{C}$ for 8 hours. After drying, the leaves were ground into fine powder. For the required concentration, 5 gm of mint powder was weighed in a polypropylene centrifuge tube and 100 ml of 50 % ethanol was added to it. Then the tube was held at room temperature ($27 \pm 1^\circ\text{C}$), for 10 min, then vortexed the sample for 10 min at high speed and then centrifuged at 5000 rpm for 10 min. The supernatant was collected in a glass test tube and the compounds were extracted again with 10 ml of the same solvent, followed by centrifugation. Both supernatants were combined and filtered through Whatman filter paper No. 42. The mixed solution was evaporated in a water bath and stored for further study. After, that the required three concentrations for the present study of mint leaf extract were prepared (0.4 %, 0.5 % and 0.6 %). In the present study concentrations have been used, based on the research carried out by (Viji *et al.*, 2015) [8], authors stated that a fish treated with 0.5% concentration of mint extract showed the best biochemical quality and extended shelf life (16–17 days), compared to 11–13 days for the control.

2.3 Analysis of extracts

2.3.1 Estimation of total phenolics

The quantification of phenolic content in mint and basil leaf extract was determined by the protocol described by (Singleton *et al.*, 1965) [10]. 0.5 ml of the extracted sample was taken in 25 ml volumetric flask containing 9 ml distilled water then, 1 ml of Folin-Ciocalteu phenol reagent was added into the flask and mixed well. After 5 min, 10 ml of 7 % Na_2CO_3 solution was added into the flask and mixed well. The solution was allowed to stand for 2 hr at room temperature and then absorbance was recorded at 760 nm using a spectrometer. The estimation of phenolic in the plant extract was calculated by using the equation ($y = 14.932x + 0.1186$, $r^2 = 0.9871$) derived from the standard curve of the known concentration of tannic acid. The result of the phenolic content of extracts was expressed in mg tannic acid equivalent/gm.

The concentration of total phenolic contents in the extract was estimated by using the following formula.

$$\text{Total phenolic content mg/gm of plant extract (T)} = \frac{C \times V}{M}$$

2.3.2 Antibacterial activity assay

Standard cultures of *Escherichia coli* (MTCC 40) and *Staphylococcus aureus* (MTCC-87) were procured from the Central Institute of Fisheries Education, Mumbai and R.P Gogate College of Arts and Science, Ratnagiri respectively.

Preparation of inoculum

After collecting a loop full of the test culture from the appropriate strains of agar slants, it was sub-cultured into new tubes with nutrient broth and kept at 37°C overnight. The bacterial suspension was added to fresh media which gives the final concentration of 10^6 cfu/ml (Harathi *et al.*, 2017) [11].

Agar well diffusion method

The antibacterial activity of plant extract was tested by the agar well diffusion method by modifying the protocol described by (Kannaiyan *et al.* 2012). Agar plates were prepared by using Mueller-Hinton agar medium. After the medium preparation, it was sterilized and cooled to solidify the medium. A bacterial suspension was swabbed with a cotton swab in three directions on Mueller-Hinton agar plates. Sterile cut pipette tips of 6 mm diameter were used to make wells. 100 μl of extracts were added into the wells. Distilled water was used as a control. The plates were incubated at 37°C for 24 hrs. The growth inhibition zones surrounding the loaded agar well were measured to record the results.

2.4 Treatment and storage study of mackerel

Indian mackerel steaks were divided into four groups of samples. One group of samples was kept as a control sample and another group of samples for mint treatment (M_1 , M_2 and M_3) were immersed in 0.4 %, 0.5 % and 0.6 % (w/v) respectively, for 30 minutes. After dip treatment, all samples were drained well for the removal of the extra extract solution. The treated and controlled Indian mackerel steak samples were packed in HDPE bags and shifted to a chiller to maintain the temperature at $0-4^\circ\text{C}$. The samples were withdrawn periodically for biochemical, microbiological and sensory quality changes. All the analyses were done in triplicate and mean values taken.

2.5 Quality assessment

2.5.1 Proximate composition

Proximate composition such as moisture%, protein%, fat% and ash% of the control, as well as treated samples, were determined by (AOAC, 2005) [12] method.

2.5.2 Sensory analysis

The attributes such as appearance, colour, odour, texture and overall acceptability were evaluated during the sensory evaluation using 9-point hedonic scales (Poste *et al.*, 1991) [13]. This enables the sensory study to be carried out with 10 panellists chosen from 10 individuals and selected based on their availability for research work. The 9-point hedonic ratings for liking with the indication of 9: like extremely, 8: like very much, 7: like moderately, 6: like slightly, 5: neither like nor dislike, 4: dislike slightly, 3: dislike moderately, 2: dislike very much

and 1: dislike extremely. Scores of separate attributes were summed up and divided by the total number of attributes to give an overall acceptability score. An overall score of four was considered as the border line for acceptability

2.5.3 Biochemical analysis

Estimation of pH was carried out by digital pH meter (EQUIPTRONICS brand, MODEL eq. 610) by the protocol given by (AOAC, 2005) [12]. Total volatile base nitrogen (TVB-N) and Trimethylamine nitrogen (TMA-N) was estimated by the microdiffusion method. Oxidative stability of the samples was assessed by determining peroxide value (AOAC, 2005) [12].

2.5.4 Microbiological analysis

The total plate count (TPC) was determined following the method given by the (USFDA, 2022) [14]. The *Escherichia coli* and *Staphylococcus aureus* of the fish steak sample was determined by the standard method given by the (USFDA, 2022) [14].

2.6 Statistical analysis

All the analysis was done in triplicate. The quality analysis results of fish steak samples were statistically analysed and presented as mean $\pm$ SD (Snedecor *et al.*, 1967) [15]. Significant differences at $p < 0.05$ between treatments and between storage days were identified through one-way ANOVA followed by post hoc analysis with 'Tukey test' respectively. The significant difference was defined at $p \leq 0.05$ (Snedecor *et al.*, 1967) [15]. The data were analysed using IBM SPSS statistics (Version 16) predictive analytics software.

3. Results

3.1 Total phenolic content of mint leaf extract (MLE)

The total phenolic content of mint extract showed an increasing trend with increasing extract concentration. Among all mint leaf extract treatments, (M_3) showed the highest total phenolic content 8.49 mg tannic acid equivalent/gm. ANOVA applied for the total phenolic content of the extract showed a significant difference between the extract concentrations ($p < 0.05$) stated in (Table 3.1).

Table 3.1. Total phenolic content of an extract

Sr.no	Treatment	Concentration of extract (%)	mg tannic acid equivalent/gm
Mint leaf extract (MLE)			
1.	M_1	0.4	8.01 ± 0.01^a
2.	M_2	0.5	8.14 ± 0.01^b
3.	M_3	0.6	8.49 ± 0.00^c

Mean $\pm$ SD^{a-c} in the same column followed by different superscript is significantly different ($p < 0.05$) ($n = 3$).

3.2 Antimicrobial activity of mint leaf extract (MLE)

The antimicrobial activity of mint extract showed an increasing trend with increasing extract concentration. Among all mint leaf extract treatments, (M_3) showed the highest zone of inhibition 6.53 mm for

Escherichia coli and 6.46 mm for *Staphylococcus aureus*. ANOVA applied for the antimicrobial activity of extract showed a significant difference between the extract concentrations ($p < 0.05$) stated in (Table 3.2).

Table 3.2. Diameter of inhibition zone (mm) of mint, basil and combined extract against fish-borne pathogen and spoilage bacteria using agar well diffusion method

Sr.no	Treatment	Concentration of extract (%)	Zone of inhibition (mm)	
			<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
			Mint leaf extract (MLE)	
1.	M_1	0.4	3.50 ± 0.1^a	3.23 ± 0.47^a
2.	M_2	0.5	5.50 ± 0.26^b	4.50 ± 0.26^b
3.	M_3	0.6	6.53 ± 0.30^c	6.46 ± 0.15^c

Mean $\pm$ SD^{a-c} in the same column followed by different superscript is significantly different ($p < 0.05$) ($n = 3$).

3.3 Proximate composition of fresh Indian mackerel fish meat

The proximate composition of fresh fish meat was determined and the values of moisture, crude protein, crude fat and ash were found to be 72.54 ± 0.24 %, 18.36 ± 0.07 %, 7.87 ± 0.17 % and 1.21 ± 0.03 % respectively.

3.4 Sensory evaluation of control and treated Indian mackerel steaks in mint leaf extract (MLE) during chilled storage period

Scores of sensory qualities of the sample are shown in (Table 3.3) during the chilled storage period. The sensory scores for the control and treated Indian mackerel steaks in mint leaf extract were continuously reduced with increased storage period. In fish steaks samples overall acceptability scores were reduced from 8.2 to 4.8, 8.6 to 4.9, 8.8 to 4.6 and 9.0 to 4.8 in M_0 , M_1 , M_2 and M_3 on their rejection day.

Table 3.3. Average values of sensory evaluation of control and Mint leaf extract (MLE) treated Indian mackerel steaks during chilled storage period

Storage days	Characteristics	Mint leaf extract (MLE) treated			
		M0	M1	M2	M3
0	Appearance	8.6 ± 0.06 ^a	8.6 ± 0.23 ^a	8.8 ± 0.24 ^b	8.9 ± 0.45 ^c
	Colour	8.3 ± 0.47 ^a	8.4 ± 0.29 ^b	8.6 ± 0.23 ^c	8.8 ± 0.15 ^d
	Odour	8.0 ± 0.54 ^a	8.1 ± 0.23 ^b	8.4 ± 0.45 ^c	8.6 ± 0.56 ^d
	Texture	8.0 ± 0.03 ^a	8.8 ± 0.45 ^b	8.8 ± 0.41 ^b	9.0 ± 0.33 ^c
	Overall acceptability	8.2 ± 0.30 ^a	8.6 ± 0.34 ^b	8.8 ± 0.34 ^c	9.0 ± 0.32 ^d
3	Appearance	7.6 ± 0.32 ^a	8.2 ± 0.21 ^b	8.4 ± 0.32 ^c	8.4 ± 0.34 ^c
	Colour	7.2 ± 0.31 ^a	7.9 ± 0.34 ^b	8.0 ± 0.45 ^c	8.2 ± 0.22 ^d
	Odour	6.9 ± 0.12 ^a	7.3 ± 0.54 ^b	7.6 ± 0.45 ^c	8.1 ± 0.25 ^d
	Texture	7.6 ± 0.23 ^a	7.9 ± 0.16 ^b	8.1 ± 0.23 ^c	8.4 ± 0.34 ^d
	Overall acceptability	7.2 ± 0.27 ^a	7.9 ± 0.34 ^b	8.1 ± 0.34 ^c	8.4 ± 0.32 ^d
6	Appearance	5.9 ± 0.34 ^a	6.0 ± 0.22 ^b	7.4 ± 0.21 ^c	8.0 ± 0.19 ^d
	Colour	5.6 ± 0.12 ^a	5.7 ± 0.14 ^b	6.9 ± 0.45 ^c	7.9 ± 0.35 ^d
	Odour	5.2 ± 0.21 ^a	5.8 ± 0.32 ^b	7.0 ± 0.34 ^c	7.4 ± 0.45 ^d
	Texture	5.2 ± 0.43 ^a	5.5 ± 0.34 ^b	6.9 ± 0.45 ^c	7.9 ± 0.32 ^d
	Overall acceptability	5.5 ± 0.11 ^a	5.7 ± 0.12 ^b	6.8 ± 0.22 ^c	7.9 ± 0.21 ^d
9	Appearance	4.8 ± 0.19 ^a	4.9 ± 0.22 ^b	5.8 ± 0.41 ^c	6.4 ± 0.39 ^d
	Colour	4.2 ± 0.39 ^a	4.9 ± 0.23 ^b	5.7 ± 0.19 ^c	5.8 ± 0.21 ^d
	Odour	4.1 ± 0.03 ^a	4.6 ± 0.34 ^b	5.8 ± 0.11 ^c	6.2 ± 0.39 ^d
	Texture	4.6 ± 0.08 ^a	4.7 ± 0.24 ^b	5.5 ± 0.23 ^c	5.7 ± 0.23 ^d
	Overall acceptability	4.8 ± 0.23 ^a	4.9 ± 0.22 ^b	5.5 ± 0.34 ^c	5.8 ± 0.45 ^d
12	Appearance	-	-	4.1 ± 0.12 ^a	4.5 ± 0.21 ^b
	Colour	-	-	4.1 ± 0.23 ^a	4.2 ± 0.34 ^b
	Odour	-	-	4.6 ± 0.45 ^a	4.8 ± 0.23 ^b
	Texture	-	-	4.2 ± 0.32 ^a	4.7 ± 0.24 ^b
	Overall acceptability	-	-	4.6 ± 0.38 ^a	4.8 ± 0.34 ^b

Mean ± SD^{a-b} in the same rows followed by different superscript is significantly different ($p < 0.05$) ($n = 10$) with respect to sampling days. M_0 (Control): Control fish steaks; M_1 : fish treated with 0.4 % Mint leaf extract; M_2 fish treated with 0.5% mint leaf extract; M_3 : fish treated with 0.6% mint leaf extract.

3.5 Biochemical evaluation of control and treated Indian mackerel steaks in mint leaf extract (MLE) during chilled storage period

3.5.1 pH

The pH values of the control and treated fish steak samples continuously increase with storage study. The changes in pH values of Indian mackerel fish steaks during storage period are given in (Fig. 1.1). In case of control samples M_0 the value of pH was increased from initial to final, 6.38 to 6.63 whereas, in M_1 , M_2 and M_3 samples, the pH increased from 6.33 to 6.55, 6.32 to 6.63 and 6.31 to 6.58 (up to 6th, 6th, 9th and 9th days) respectively.

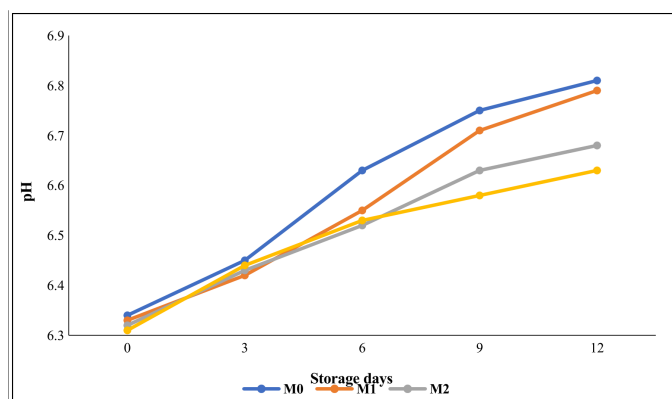


Figure 1.1. Changes observed in the pH content of Indian mackerel steaks stored at chilled temperature; Values represent mean ± SD ($n = 3$);

M_0 : Control; M_1 : fish treated with 0.4 % Mint leaf extract; M_2 : fish treated with 0.5% mint leaf extract; M_3 : fish treated with 0.6% mint leaf extract.

3.5.2 Trimethylamine nitrogen (TMA-N)

The observed values of TMA-N were stated in (Fig. 1.2). The content of TMA-N of control and treated samples showed a gradual increase with increasing storage days during the chilled storage. Initial TMA-N values were recorded 2.36, 2.32, 2.38 and 2.31 mg/100gm for M_0 , M_1 , M_2 and M_3 respectively. The TMA-N content was increased to 8.56, 8.22, 9.23 and 8.78 mg/100gm for M_0 , M_1 , M_2 and M_3 respectively (6th, 6th, 9th and 9th days) at the end of the storage study. The control fish steak sample recorded a higher value of TMA-N as compared to the treated sample steaks.

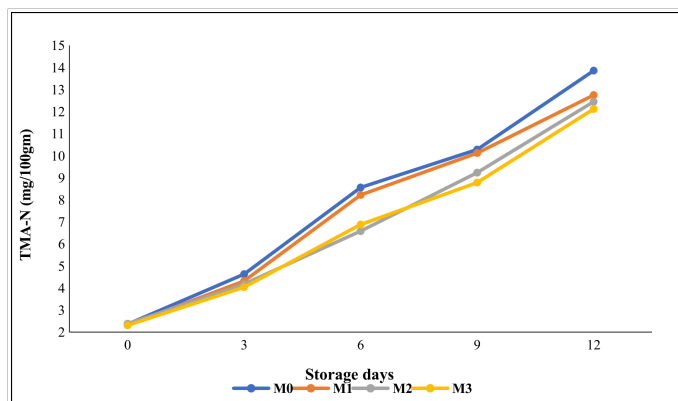


Figure 1.2. Changes observed in the TMA-N content of Indian mackerel steaks stored at chilled temperature; Values represent mean $\pm$ SD ($n = 3$); M_0 : Control; M_1 : fish treated with 0.4 % Mint leaf extract; M_2 : fish treated with 0.5% mint leaf extract; M_3 : fish treated with 0.6% mint leaf extract.

3.5.3 Total volatile basic nitrogen (TVB-N)

The observed values of TVB-N were stated in (Fig. 1.3). The content of TVB-N of control and treated samples showed a gradual increase with increasing storage days during the chilled storage. Initial TVB-N values were recorded 11.43, 11.83, 11.78 and 11.73 mg/100gm for M_0 , M_1 , M_2 and M_3 respectively. The TVB-N content was increased to 24.61, 23.67, 25.07 and 24.19 mg/100gm for M_0 , M_1 , M_2 and M_3 respectively (6th, 6th, 9th and 9th days) at the end of the storage study. The control fish steak sample recorded a higher value of TVB-N as compared to the treated sample steaks.

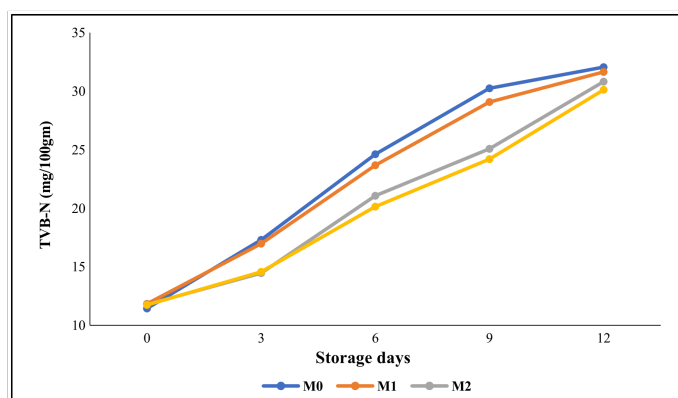


Figure 1.3. Changes observed in the TVB-N content of Indian mackerel steaks stored at chilled temperature; Values represent mean $\pm$ SD

($n = 3$); M_0 : Control; M_1 : fish treated with 0.4 % Mint leaf extract; M_2 : fish treated with 0.5% mint leaf extract; M_3 : fish treated with 0.6% mint leaf extract.

3.5.4 Peroxide value (PV)

The observed values of PV were stated in (Fig. 1.4). The peroxide value of the control and treated samples gradually increased with increasing storage period. Initial peroxide values for M_0 , M_1 , M_2 and M_3 were recorded 2.64, 2.63, 2.40 and 2.20 of meq of O_2 /kg respectively. The peroxide value was increased to 7.55, 6.88, 9.29 and 8.66 meq of O_2 /kg for M_0 , M_1 , M_2 and M_3 respectively (6th, 6th, 9th and 9th days) at the end of the storage study. The control fish samples recorded a high peroxide value as compared to the treated samples.

The ANOVA test results indicated that the pH content, TMA-N content, TVB-N content and PV content for M_0 , M_1 , M_2 and M_3 of mint leaf extract treated fish steaks sample showed a significant difference ($p < 0.05$) and it was confirmed by Tukey test results.

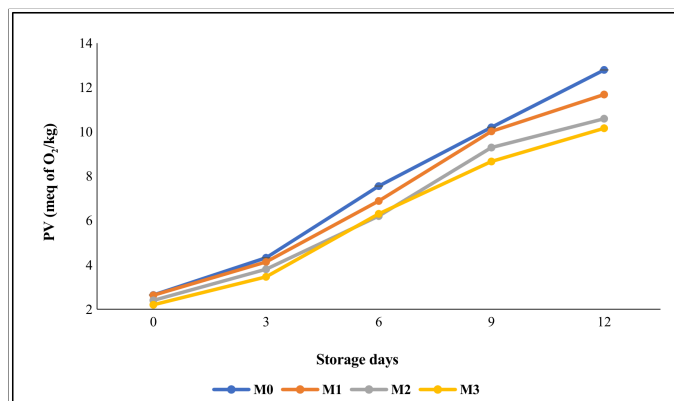


Figure 1.4. Changes observed in the PV content of Indian mackerel steaks stored at chilled temperature; Values represent mean $\pm$ SD ($n = 3$); M_0 : Control; M_1 : fish treated with 0.4 % Mint leaf extract; M_2 : fish treated with 0.5% mint leaf extract; M_3 : fish treated with 0.6% mint leaf extract

3.5.5 Microbiological evaluation of control and treated Indian mackerel steaks in mint leaf extract (MLE) during chilled storage period

3.5.5.1 Total plate count

The TPC (Total plate count) changes in control and treated fish steak samples are given in (Fig. 1.5). The total plate count (cfu/gm) increased with storage period in all treated and control samples. The TPC was increased from 2.48 to 4.26 log cfu/gm in control (M_0) sample at the 6th day of storage period and for M_1 , M_2 and M_3 were increased from 2.47 to 3.93 log cfu/gm, 2.45 to 4.60 log cfu/gm and 2.43 to 4.28 log cfu/gm at 6th, 9th and 9th days of storage period.

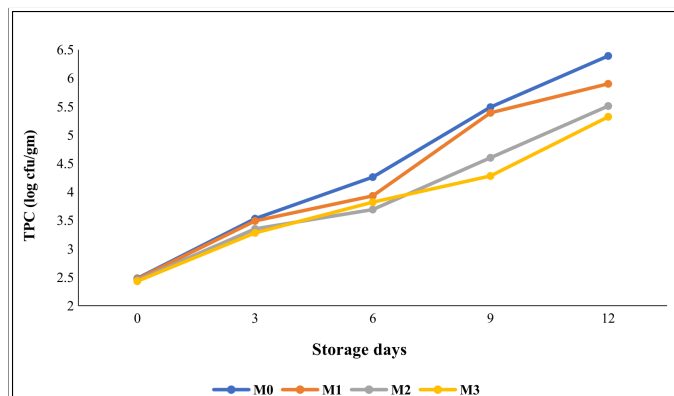


Figure 1.5. Changes observed in the TPC content of Indian mackerel steaks stored at chilled temperature; Values represent mean $\pm$ SD ($n = 3$); M_0 : Control; M_1 : fish treated with 0.4 % Mint leaf extract; M_2 fish treated with 0.5% mint leaf extract; M_3 : fish treated with 0.6% mint leaf extract

3.5.5.2 Escherichia coli

The pathogenic bacteria *Escherichia coli* was not detected in the mint leaf extract treated fish steaks samples with different concentrations.

3.5.5.3 Staphylococcus aureus

The pathogenic bacteria *Staphylococcus aureus* was not detected in the mint leaf extract treated fish steaks samples with different concentrations.

4. Discussion

4.1 Total phenolic content of mint leaf extract

The phenolic content in mint leaf extract was observed to be 8.01 ± 0.01 , 8.14 ± 0.01 and 8.49 ± 0.00 mg tannic acid equivalent/gm for M_1 , M_2 and M_3 respectively, as shown in (Table 4.1 and Figure 4.1). Atanassova et al. (2011) [16] stated the results of the total phenolic

content of lemon balm (*Melissa officinalis*), sage (*Salvia officinalis*), and mint (*Mentha piperita*) were (48.86 mg GAE/100gm), (27.94 mg GAE/100gm), and (45.45 mg GAE/100gm) respectively. Fezea *et al.* (2017) [17] stated the phenolic content present in control, white soft cheese + 1% AMLE (aqueous mint leaf extract), white soft cheese + 2% AMLE (aqueous mint leaf extract) and white soft cheese + 3% AMLE (aqueous mint leaf extract) were (24.5 ± 0.7), (76.5 ± 2.1), (82.5 ± 2.1) and (96.0 ± 4.2) GAE/100gm respectively. Pandian *et al.* (2017) [18] studied the total phenolic content present in curry leaf, wild mint, and coriander and stated the results as (70.12 ± 2.0 mg GAE/gm), (29.04 ± 1.6 mg GAE/gm), and (24.02 ± 2.4 mg GAE/gm) respectively. Ping *et al.* (2023) [19] evaluated the methanolic extract of stir-fried mint leaves had the highest total phenolic content (315.09 mg/gm), followed by oven-dried (60 °C) (273.20 mg/gm), oven-dried (40 °C) (234.42 mg/gm), and air-dried mint leaves (128.33 mg/gm). It has been reported that phenolic compounds of plants are present in different bound status depending on species, effective processing steps for liberating phenolic compounds from various plants may be different (Jeong *et al.*, 2004) [20].

4.2 Antimicrobial activity of mint leaf extract

The antimicrobial activity of mint leaf extract of M_1 , M_2 and M_3 concentrations showed a zone of inhibition of 3.50, 5.50 and 6.53 mm for *Escherichia coli* and 3.23, 4.50 and 6.46 mm for *Staphylococcus aureus*, showed in Table 4.2 and Fig 4.4. In the present study, a significant difference has been observed among all the concentrations of mint leaf extract. Similarly, with slight variation Hammadi *et al.* (2021) [21] evaluated the antimicrobial properties of ethanolic extracts from *Mentha piperita* against *Escherichia coli* and *Staphylococcus aureus*. They observed the results of *Staphylococcus aureus* for *M. piperita* made crude extract in three dilutions (0.25 mg/5 ml), (0.25 mg/10ml) and (0.25 mg/15ml) and the inhibition zone measured for those dilutions were 10 mm, 8 mm and 6 mm in diameter, respectively and for *E. coli*, the inhibition zone measured for that same dilutions was 10 mm, 8 mm and 6 mm in diameter, respectively. The present study revealed that mint leaf extract showed inhibition against *Escherichia coli* and *Staphylococcus aureus* due to the flavonoids from plant extracts, which have been found to possess antioxidants and antimicrobial properties in various studies (Biswas *et al.*, 2012) [9].

4.3 Sensory evaluation

Scores of sensory evaluations of the samples obtained during the chilled storage period are shown in (Table 3.3). Scores of sensory evaluations of the samples obtained during the chilled storage period are shown in Table 4.6 and Fig 4.9, 4.10, 4.11, 4.12 and 4.13. The sensory scores were given by panellist's using nine - point hedonic scale for control (M_0) and the mint treated fish steaks (M_1 , M_2 and M_3) immersed in 0.4, 0.5 and 0.6 % MLE respectively. The sensory scores for the control and mint treated Indian mackerel steaks were continuously reduced with an increase in storage period. Changes in the initial to final overall acceptability scores of Indian mackerel during the chilled storage period were found to decrease from 8.6 to 4.8, 8.6 to 4.9, 8.8 to 4.6 and 8.9 to 4.8 in M_0 , M_1 , M_2 and M_3 , respectively on their rejection day. The overall score declined rapidly from initial to final in control samples as compared with the fish treated with mint leaf extract. A similar, trend has also been observed by Viji *et al.* (2015) [8]. In the present study, control and mint treated (0.4, 0.5 and 0.6 %) samples were considered to be acceptable for consumption up to 6 days, 6 days, 9 days and 9 days, respectively during chilled storage. The study indicated that treatment with mint leaf extract was effective in retarding the spoilage and extending the shelf life of Indian mackerel compared to the control samples. The primary phenolic content in mentha species, luteolin, eriocitrin, caffeic acid, and rosmarinic acid, may be responsible for the preservation effect of the mint leaf extract (Padmini *et al.*, 2010) [22].

4.4 Biochemical evaluation

4.4.1 Changes in pH

The initial post-mortem pH varies from species to species, catching ground, and due to the season variation (Viji *et al.*, 2015) [8]. The fish is acceptable up to pH 6.8 and spoiled fish with pH above 7.0 (Huss, 1988) [23]. A pH higher than 7.9 leads to loss of tissue quality and reduces the acceptability of the product due to spoilage according to Aberoumandand *et al.* (2020) [24].

In the present study, fresh Indian mackerel shows a pH range from 6.38 to 6.31, similar results were reported by (Viji *et al.*, 2015) [8]. All control and treated samples of Indian mackerel steaks showed an increasing trend in the pH content from the commencement of the experiment up to the end (Table 4.7 and Fig. 4.14). The pH increased from initial to final day from 6.38 to 6.75, 6.33 to 6.71, 6.32 to 6.68 and 6.31 to 6.63 for control and MLE treated (M_1 , M_2 and M_3) samples on their rejection day 9th, 9th, 12th and 12th days of storage, respectively. An increase in pH over the storage period is attributed to the production of amines and other volatile bases by the autolytic and microbial action on protein and other compounds (Binsi *et al.*, 2009) [25]. In the present study, on the 12th day, M_3 shows a significant difference from the control, M_1 and M_2 samples. A significantly low pH value was observed in (M_3) treated samples at the end of rejection day during chilled storage. This may be due to the antimicrobial and antioxidant characteristics of mint leaf extract. Similar results were observed by Viji *et al.* (2015) [8], as pH of all the samples increased and reached to 6.58, 6.59 and 6.61 for control, citrus extract and mint leaf extract treated samples on their rejection day 13th, 15th and 17th days of storage, respectively.

4.4.2 Changes in trimethylamine nitrogen (TMA-N)

TMA-N is a crucial indicator of deterioration for marine fish, trimethylamine oxide (TMAO) is the source of TMA-N and is essential to marine fish for osmoregulation (Gopakumar, 2002) [26]. Enzymes reduce TMAO to TMA during spoilage (Viji *et al.*, 2015) [8]. According to Teskeredžić *et al.* (1987) [27], the offensive fishy odour occurs when the TMA-N concentration of fish exceeds 10 mg/100 gm. TMA-N between 10-15 mg/100gm muscle is considered as the limit of acceptability for round, whole chilled fish (Jeyakumari *et al.*, 2018) [28].

In the present study, all samples of Indian mackerel steaks showed an increasing trend in the TMA-N from commencement of experiment up to the end of the experiment (Table 4.8 and Fig. 4.15). The TMA-N values of control samples increased with storage period from 2.36 to 10.28 mg/100gm on 9th day of rejection. Whereas, in M_1 , M_2 and M_3 samples the TMA-N values increased from 2.32 to 10.12, 2.38 to 12.45 and 2.31 to 12.12 mg/100gm on 9th, 12th and 12th day of rejection, respectively. At 0 day, all the samples showed a similar level of TMA-N, no significant difference were found. Similarly, Viji *et al.* (2015) [8] also observed no significant difference among the samples and also observed all the samples showed similar levels of TMA-N on 1st day of storage. In the present study, on the 6th day control showed a higher level of TMA-N ($p < 0.05$) than the MLE treated samples. TMA-N level in the treated samples remained lower than that of the control samples; however significant difference ($p < 0.05$) was observed on 6th and 9th day. Also, on the 9th day, M_3 showed a significant difference from the control, M_1 and M_2 samples. The significantly low TMA-N value was observed in (M_3) treated samples till the end of storage days. On the 6th, 6th, 9th and 9th day of storage period control and all the treated samples were within the acceptable limit TMA-N. Results of TMA-N indicated that the mint leaf extract was efficient in preventing bacteria from decarboxylating trimethyl amine oxide into trimethyl amine Viji *et al.* (2015) [8]. The results of the present study are in agreement with those of Mohan *et al.* (2011) [29] who reported that the initial TMA-N content was 6.01 mg N/100gm, which increases with the storage period and the control samples exhibited a significantly higher increase in trimethylamine nitrogen ($p < 0.05$).

4.4.3 Changes in total volatile basic nitrogen (TVB-N)

TVB-N measures the number of volatile bases formed from solubilized nitrogen derivatives (Jeyakumari *et al.*, 2018) [28]. According to Sikorski *et al.* (2020), TVB-N content at a level of 30 mg/100gm is indicated as spoilage of fish. TVB-N is the result of bacterial deterioration and the activity of endogenous enzymes, and its concentration is frequently used as an indicator to evaluate the shelf life and quality of goods (EEC, 1995) [30].

In the present study, all samples of Indian mackerel fish steaks showed an increasing trend in the TVB-N from commencement of experiment up to the end of the experiment (Table 4.9 and Fig. 4.16). The TVB-N values of control samples increased with the storage period from 11.43 to 30.24 mg/100gm on 9th day of rejection. Whereas, in mint leaf extract treated samples, in M_1 , M_2 and M_3 the TVB-N increased from 11.83 to 29.07, 11.78 to 30.81 and 11.73 to 30.11 mg/100gm on 9th, 12th and 12th day of the rejection, respectively. TVB-N content of fresh fish was 11.43 mg/100gm in agreement with the initial fresh fish value reported by Viji *et al.* (2015) [8]. Similarly, Viji *et al.* (2015) [8] stated that the TVB-N values increased significantly ($p < 0.05$) from an initial value of 11.5–12.02 mg % to a final value of 25.18, 26.64 and 26.91 mg % respectively, for control, citrus extract and mint leaf extract samples on their respective rejection days. In the present study, at 0 day, all the samples showed a similar level of TVB-N, no significant difference was found, but samples showed an increasing range of TVB-N as the storage day increased. Control samples had significantly higher levels ($p < 0.05$) of TVB-N 30.24 mg/100gm than treated samples on the 9th day of storage. Similar results were stated by, Mohan *et al.* (2011) [29] that control samples exhibited a significantly ($p < 0.05$) higher level of TVB-N 34.6 mg/100gm, than treated samples on the 5th day of storage. (Padghan *et al.*, 2025) [31] Also stated combined extract (Mint leaf extract + Basil leaf extract) helps in controlling the biochemical indices such as TVB-N during the chilled storage study and extended the shelf life of Indian mackerel steaks up to 15 days.

Also, on the 9th day, M_3 showed a significant difference from the control, M_1 and M_2 samples. The significantly low TVB-N value was observed in M_3 treated samples till the end of storage study. On the 6th, 9th and 9th day of the storage period control and all the treated samples (M_1 , M_2 and M_3) were within the acceptable limit TVB-N.

4.4.4. Changes in peroxide value (PV)

Lipids in fish are susceptible to oxidation and peroxide value measures the amount of hydroperoxides formed i.e., the primary lipid oxidation products in fish muscle (Viji *et al.*, 2015) [8]. As per acceptable standards, good quality fish contain peroxide value below 10 meq of O_2 /kg (Gopakumar, 2002) [26].

In the present study, the effect of mint leaf extract on the changes in peroxide value of chilled stored Indian mackerel steaks was studied. All samples of Indian mackerel steaks showed increasing trend in the peroxide value from commencement of experiment up to the end of the experiment (Table 4.10 and Fig. 4.17). The peroxide values of control samples increased with storage period from 2.64 to 10.20 meq of O_2 /kg on 9th day of rejection. Whereas, in M_1 , M_2 and M_3 samples the peroxide value increased from 2.63 to 10.02, 2.40 to 10.59 and 2.20 to 10.16 meq of O_2 /kg on 9th, 12th and 12th day of rejection, respectively. At day 0, there was no significant difference between the peroxide values of different treatment samples was observed. As storage progressed, control and treated samples showed a progressive increase in peroxide value. Similarly, Viji *et al.* (2015) [8] also found no significant difference between the peroxide value of different treatment samples on first day, although they stated that maximum value recorded for peroxide value was 5.54, 4.54 and 5.52 meq O_2 /kg samples for control, citrus extract and mint leaf extract samples, respectively. Bensid *et al.* (2013) [32] also stated the peroxide values at the end of the storage study was 16.20, 7.76, 10.87 and 6.44 meq O_2 /kg for control, thyme (0.04% w/v), oregano (0.03% w/v), and clove (0.02% w/v) respectively. Similarly, (Bhor *et al.*, 2026) [33] also reported that peroxide content increased with increased storage time.

In the present study, significant difference between the peroxide value of control and treated samples observed after 9 days of storage. Similarly, Eskandari *et al.* (2014) [34] also observed a substantial ($p < 0.05$) difference in peroxide value between controls and samples treated with Cumin, Caraway and Cumin + Caraway after 15 days of storage. In the present study, results indicated that highest peroxide value was recorded in control samples. Whereas fish steaks treated with mint leaf extracts showed significantly poor peroxide production ($p < 0.05$). As stated by Turhan *et al.* (2009) [35], phenolic antioxidants do not act as oxygen absorbers they inhibit the formation of fatty acid free radicals, which do react with or absorb oxygen in the auto-oxidation process.

4.5 Microbiology evaluation

Bacterial growth and activity are mostly responsible for the primary changes in fish freshness, such as the undesirable changes in food attributes like flavour, odour, and colour (Huss, 1988; Connell, 1990) [23, 36]. The APC (Aerobic Plate Count) value of 5×10^5 cfu/gm considered acceptable limit for raw/chilled/frozen fin fish; While, the APC of 10^7 cfu/g indicates hazardous quality for human consumption (FSSAI, 2017). According to the Sallam *et al.* (2007) [37], the predominant category of microorganisms accountable for the aerobic deterioration of fish held at chilled temperatures is gram-negative psychrotrophic bacteria.

In the present study, all samples of Indian mackerel steaks showed an increasing trend in the TPC (log cfu/g) till the end of the chilled storage study (Table 4.11 and Fig. 4.18). The TPC was increased from initial to final day, 2.48 to 5.49 log cfu/gm in control (M_0) sample at the 9th day of storage period and for M_1 (0.4%), M_2 (0.5%) and M_3 (0.6%) were found to be increased from 2.47 to 5.39 log cfu/gm, 2.45 to 5.51 log cfu/gm and 2.43 to 5.32 log cfu/gm at the end of their rejection day on 9th, 12th and 12th day of storage, respectively. In the present study, at 0 day no significant difference among the treated and control was observed. As the storage period increased, microbial load also increased. Similar, trend observed in the study of Viji *et al.* (2015) [8]. In present study at 6th day of storage, significant difference of TPC between the control and treated samples was observed in agreement with the result of observed by Raisi *et al.* (2015) [7]. The author stated that for control samples (control), TPC reached a value higher than acceptable limit on 6th day of storage (7.16 log cfu/gm). Viji *et al.* (2015) [8] stated the results, as its control sample attained a count of 7.13 log cfu/gm on 11th day of storage. In the present study, significant difference between the control and treated samples was observed on the 12th day of storage. On 12th day M_3 and M_2 showed a significant difference from control and M_1 sample. The significant ($p < 0.05$) low TPC content was observed in M_3 treated samples on the rejection day during chilled storage study. The significant reduction in TPC observed in the treated samples of mackerel may be attributed due to the antibacterial effect of plant extracts on aerobic spoilage bacteria, resulting in 3 to 4 days extension of microbiological shelf life compared to control.

In the present study, the pathogenic bacteria *S. aureus* and *E. coli* were not detected during the chilled storage study. Similarly, Phadke *et al.* (2012) [38], studied on the quality changes of seer fish and stated that *E. coli* and *S. aureus* were not present throughout the chilled and frozen storage period. In the present study, the antibacterial activity of mint leaf extract has been studied against *S. aureus* and *E. coli* which confirms that mint leaf extract may have a potential effect on the bacteria; this is in agreement with the results of Sugandhi *et al.* (2011) [39], stated that mint (*M. arvensis*) extract has potential antibacterial activities against *S. aureus*, *E. coli*, *P. aeruginosa*, *S. flexneri*, *K. pneumonia*.

5. Conclusion

The present study revealed a significant effect of mint leaf extract (MLE), which helps in controlling the biochemical indices such as TVB-N, TMA-N and PV during the chilled storage study and extended the shelf life of Indian mackerel steaks up to 9 days, respec

tively. The study also showed, a positive preservative effect of mint leaf extract; hence, mint extract can be considered a potential natural preservative in fish and fishery products.

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