

## Antioxidant and antibacterial activities of dill extracts and their preservative effect on mackerel fillets during refrigerated storage

Sathish Kumar Kannaiyan<sup>1</sup>, Janarthanan Gunasekaran<sup>2</sup>, Nagalakshmi Kannuchamy<sup>3</sup>, Madonna T Thachil<sup>3</sup> and Venkateshwarlu Gudipati<sup>3\*</sup>

<sup>1</sup>Office of the Assistant Director of Fisheries (South), Ramanathapuram - 623 501, Tamil Nadu, India

<sup>2</sup>Office of the Assistant Director of Fisheries (DMS Campus), Chennai- 600 006, Tamil Nadu

<sup>3</sup>Fishery Resources, Harvest and Post-Harvest Management Division, Central Institute of Fisheries Education, Mumbai- 400 061, Maharashtra, India

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In order to develop a natural extract using no organic solvent for extension of shelf life of mackerel fillets, juices were prepared from raw, water bath boiled (WBB) and microwave oven boiled (MOB) dill (*Anethum sowa* Kurz) plant. The MOB dill juice showed the highest percentage of inhibition in DPPH radical scavenging activity and also had high total phenolic content revealing the role of phenolic compounds in their antioxidant activity. The antibacterial activities of the dill juices were evaluated against 13 fish borne pathogens and fish spoilage bacteria using micro broth dilution methods. Among the dill juices, MOB dill juice showed lowest MIC and MBC values in the range of 250 to 500  $\mu\text{L/mL}$  and 250 to 1000  $\mu\text{L/mL}$ , respectively, demonstrating their potential antibacterial activity. Among the three dill juice extracts studied, MOB dill juice was selected for preservation of mackerel fillets based on its antioxidant and antibacterial activity exhibited against fish borne pathogens and fish spoilage bacteria. The MOB dill juice extended the shelf life of mackerel fillets by three days demonstrating its capacity as an excellent natural antioxidant and antibacterial agent which could be an effective alternative to synthetic antioxidant and antibacterial agents.

**Keywords:** Dill, *Anethum sowa* Kurz, DPPH, FRAP, Phenolics, Antibacterial activity, Fish, Mackerel fillets.

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### Introduction

The Indian mackerel, *Rastrelliger kanagurta* Cuvier is a pelagic shoaling fish widely distributed in the Indo-west Pacific region. It is one of the major marine fishery resources of India and a highly perishable food item. Though chilling is considered as the most effective method for keeping fresh fish quality, refrigeration is more appealing to a common man since the former is mainly used in retail shops and the latter both in retail shops and households. However, fish deterioration during low temperature storage has been reported mainly due to lipid oxidation and microbial spoilage<sup>1,2</sup>. The use of antioxidants suppresses the lipid oxidation by reducing the availability of metal catalysts and quenching the radicals in the system, leading to the termination of oxidative radical chain reactions<sup>3,4</sup>. The most widely used antioxidants in food at present are butylated hydroxyl toluene, butylated hydroxyl anisole, propyl gallate and tertiary butyl hydro

quinone. However, due to unstable nature and possible adverse side effects of these synthetic antioxidants, the demand for novel natural antioxidant sources has greatly increased<sup>4,5</sup>.

Besides the quality and nutritive problems as a result of lipid oxidation during storage, there is also the risk of food poisoning, if the fish gets contaminated with pathogenic bacteria either during handling, processing or storage. However, the indiscriminate use of antibiotics against these microorganisms triggers the bacteria to develop resistance against these antibiotics<sup>6</sup> and cause adverse side effects like allergy in humans<sup>7</sup>. Not only the antibiotics but also the chemical preservatives used in food also pose adverse side effects to human health. Many of the chemical preservatives used by large scale industrial food manufacturers are inconclusively researched and potentially harmful<sup>8</sup>. This invariably boosted the interest towards the development of new types of effective, nontoxic, bioactive compounds for food preservation, such as extracts of spices, herbs, fruits and vegetables for food preservation which exhibit a broad range of biological activities like

Correspondent Author:  
E-mail: venkaticar@rediffmail.com

antibacterial, anti-inflammatory, antifungal and antioxidant activities<sup>9-13</sup>.

Dill (*Anethum sowa* Kurz), a green herb is reported to have antibacterial and antioxidant properties<sup>14-16</sup>. However, most of the reports about antibacterial and antioxidant effects of dill are rooted on its organic solvent and essential oil extracts<sup>12,17,18</sup> and are derived from *in vitro* studies instead of real food systems. The organic solvent extracts may pose residue problems and difficulty in incorporation into aqueous food systems. The use of essential oil is nonetheless limited because of its susceptibility to oxidation, volatility, thermal instability<sup>19,20</sup> and immiscibility with aqueous foods. Alternative extraction methods using no organic solvents would be essential to avoid these problems. In this background, the present study was aimed at the evaluation of the antioxidant and antibacterial properties of different types of dill extracts and their suitability in extending the shelf life of mackerel fillets during refrigerated storage.

## Materials and Methods

### Preparation of dill extracts

Dill was purchased from local market and washed in distilled water. An amount of 100 g dill was ground for 2 min without adding water or other solvents. Three types of dill extracts were prepared from the ground mixture; raw extract was obtained by simple sieving/filtering of the ground mixture through a fine cotton cloth upon pressure; water bath boiled (WBB) extract was prepared by boiling the ground mixture (100 g) for 30 min followed by sieving. To prepare microwave oven boiled (MOB) extract, 100 g of ground mixture was cooked in a microwave oven for 2 min and sieved. The three extracts were stored under refrigeration at 4 °C.

### Determination of antioxidant activities

#### DPPH radical scavenging activity

The antioxidant activity of dill extracts were measured in terms of hydrogen-donating or radical scavenging ability, using the stable DPPH radical. A volume of 150 µL of dill extract of five different concentrations (4, 8, 12, 16 and 20 %) was made by diluting with distilled water and put into screw-cap test tubes. Methanolic DPPH ( $6 \times 10^{-5}$  M) of 2 mL volume was added to it. The mixtures were then shaken for 1 min and then placed in dark for 30 min. The decrease in absorbance at 517 nm was determined using a UV-spectrophotometer with

methanol used as blank. Methanolic DPPH without added dill extract was considered as control and its absorbance was measured. The percentage inhibition of the DPPH radical was calculated as per the formula<sup>21</sup>:

$\% I = [(A_B - A_S) / A_B] \times 100$ , where, I = DPPH inhibition (%),  $A_B$  = absorbance of control (t = 0 min) and  $A_S$  = absorbance of tested sample at the end of the reaction (t = 30 min).

The concentration of sample required for the conversion of the half of the DPPH radicals to their more stable molecular counterparts is known as  $IC_{50}$ , which is measured as the amount of sample necessary to decrease the absorbance of DPPH by 50 %.

#### Ferric reducing antioxidant power (FRAP) assay

The ferric reducing ability of different extracts was determined according to the method previously described with some modifications<sup>22</sup>. The FRAP reagent was prepared freshly and warmed to 37 °C in a water bath prior to use. A volume of 20 µL was taken into screw-cap test tube from different concentration (20, 40, 60, 80 and 100 %) of extracts and 2 mL of the FRAP reagent was added into each tube and mixed well. The absorbance of the reaction mixture was then recorded using UV-spectrophotometer at 593 nm after 4 min. The standard curve was constructed using iron (II) sulphate solution (100-2000 µM) and the results were expressed as µ mol Fe (II)/g.

#### Total phenolics

A volume of 30 µL of the dill extract was taken in a test tube and made up to 3 mL with distilled water. Aliquot of 0.5 mL of Folin-Ciocalteu reagent was added to the test tube. After 3 min, 2 mL of 20 % sodium carbonate was added and mixed well. The tubes were then placed in a boiling water bath for 1 min, cooled and the absorbance was measured using UV spectrophotometer at 650 nm against a reagent blank. The standard curve was plotted using catechol as standard. The amount of total phenolics was calculated as catechol equivalents in mg/100 g<sup>23</sup>.

#### Determination of antibacterial properties

##### Microorganisms and culture

The standard cultures of bacterial strains (MTCC, Chandigarh, India) and a few lab isolates were used for the study. The details of the bacterial strains are as follows: *Pseudomonas aeruginosa* (MTCC-4676), *Aeromonas hydrophila* (lab isolate), *Bacillus subtilis* (MTCC-121), *Escherichia coli* (MTCC-40), *Salmonella typhi* (MTCC-3220), *Yersinia enterocolitica* (MTCC-859), *Vibrio cholerae* (MTCC-

3904), *Staphylococcus aureus* (MTCC-87), *Listeria monocytogenes* (MTCC-657), *Bacillus licheniformis* (MTCC-429), *Bacillus cereus* (lab isolate), *Bacillus pumilus* (lab isolate) and *Micrococcus sp.* (lab isolate). All the cultures were maintained on Nutrient Agar slants at 4 °C.

Each bacterial strain from stock culture was inoculated in 5 mL of Nutrient Broth and incubated overnight at room temperature. It was then centrifuged at 10,000 rpm for 5 min. The pellets were washed twice with Phosphate Buffer and re-suspended in 0.85 % saline water. The bacterial concentration was adjusted to 0.5 McFarland standards ( $10^8$  cfu/mL) by turbidity measurement at 600 nm in UV-spectrophotometer. A 1:10 dilution of the cell suspension ( $10^7$  cfu/mL) was used for the test.

#### **Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)**

The MIC of dill extract was determined by Micro Broth Dilution method according to the National Committee for Clinical Laboratory Standard guidelines<sup>24</sup> using a 96 well tissue culture test plate. Aliquots of 100 µL sterilized Mueller Hinton Broth was added in each well of tissue culture plate followed by 100 µL of dill extract. A serial dilution was performed to obtain the concentrations in the range from 1000 µL/mL to 3.75 µL/mL. Aliquots of 10 µL inoculum of each bacteria was added to the wells and incubated at ambient temperature for 24 h. A positive control containing only inoculum without dill extract and a negative control containing only dill extract without inoculum were included on each plate. The MIC was determined by Tetrazolium based colorimetric method. To each well, 10 µL of 1 % Triphenyl Tetrazolium Chloride was added. Plates were kept at 30 °C. A colour change of pinkish red colour was taken as positive. The lowest concentration of dill extract inhibited the bacterial growth in the wells was considered as MIC. Later, a loop full of inoculum taken from wells having no bacterial growth was streaked on Mueller Hinton Agar (MHA) plates and incubated for further 24 h at 37 °C. The lowest concentration of dill extract inhibiting the bacterial growth on MHA plates was considered as MBC.

#### **Storage study**

##### **Treatment of mackerel fillets with dill extract**

Mackerel fillets were dipped in 10 and 20 % of MOB dill extracts for 30 min at room temperature and the excess liquid was drained off. The fillets given no

dip treatment was considered as control. Both the control and treatment samples were then placed in sterile polythene bags and stored at a refrigerated temperature of 4 °C. The samples in triplicates were randomly removed from each treatment group periodically (0, 3, 6, 9 and 12 days) and the biochemical and microbial characteristics were determined.

#### **Total plate count (TPC)**

The TPC was estimated by spread plate technique. Fish sample of 10 g was homogenized aseptically with 90 mL saline in a Stomacher (Seward Stomacher 80) for 60 seconds. Using a sterile pipette, 1 mL of the supernatant was aseptically transferred into a 9 mL saline tube and mixed well. Further dilutions were prepared. Aliquots of 0.1 mL each of the appropriate dilutions was spread on the plate. The plates were incubated at 37 °C for 24 h. The average count of individual bacterial colonies in the triplicates was taken and the count was calculated as cfu/g of the sample.

$$\text{cfu/g} = \frac{\text{Average count} \times \text{Dilution factor} \times 10}{\text{weight of the sample}}$$

#### **Thiobarbituric acid reactive substances (TBARS)**

Fish sample of 10 g was blended thoroughly with 50 mL of distilled water. The mixture was washed out into a 250 mL round bottom flask with 47.5 mL distilled water and 2.5 mL of 4 N HCl. The flask was then connected to a distillation unit and heated by an electric mantle in such a way that 50 mL of the distillate was collected in 10 min. The distillate of 5 mL volume was taken in a test tube and was added with 5 mL TBA reagent. The tubes were then placed in a boiling water bath for 35 min. After cooling, the optical density was measured against the reagent blank at 538 nm by a UV-spectrophotometer. The amount of TBARS was expressed as mg malonaldehyde (MDA)/kg sample<sup>25</sup>.

#### **Sensory evaluation**

The sensory evaluation of mackerel fillets was done based on characterization and differentiation of the various organoleptic characteristics such as appearance, odour, flavour, taste, consistency and overall acceptability. Score was given on a 10-point hedonic scale by a sensory panel consisting of 6 regular members, as per the guidelines given in IS: 6273[II]-1971. An overall acceptance score was calculated as an average of all scores. The score of 6 and above was considered as acceptable.

### Statistical analysis

The results were expressed as Mean±Standard Error and the One-way ANOVA was performed to compare the results of different treatments. The significant difference between the treatments was determined by Tukey's HSD Test and the level of significance was set up at  $p \leq 0.05$ . The statistical package, SPSS (Version 16, SPSS Inc, Chicago, IL) was used for data analysis.

### Results

#### Antioxidant activity of dill extracts

The DPPH assay is a widely used method to evaluate antioxidant activity in a relatively short time compared to the other methods. The decrease in absorbance occurs when the DPPH radical accepts an electron or hydrogen from an antioxidant and becomes a stable diamagnetic molecule. It was observed from Table 1 that the extracts exhibited varying degrees of DPPH radical scavenging capacities and effective concentration among the treatments and across different dill concentrations.

The IC<sub>50</sub> values were lower in WBB and MOB dill extracts compared to the raw extract. Both WBB and MOB dill extracts showed significantly highest DPPH radical scavenging effects at 16 and 20 % concentrations ranging from 88.23±1.92 % to 92.73±1.04 %. However, the highest DPPH radical scavenging effect was demonstrated by MOB dill extract at 20 % concentration. Another measure of the antioxidant potential, Ferric reducing activity, was estimated based on the ability of dill extracts to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II) complex by the antioxidants present in them. Among the three extracts, MOB dill extract showed significantly highest ferric reducing activity (254.37±1.25) at a dill concentration of 100 % (Table 1). MOB dill extract at the concentration of 100 % also showed a significantly highest content of phenolics (Table 1). Both the ferric reducing ability and total phenolic contents of raw ( $R^2=0.981$ ,  $P<0.01$ ), WBB ( $R^2=0.962$ ,  $P<0.01$ ) and MOB ( $R^2=0.972$ ,  $P<0.01$ ) dill extracts (Table 2) showed a linear positive correlation.

Table 1— Antioxidant activity of dill extracts at different concentrations

Dill extract	Percentage inhibition of the DPPH radical					IC <sub>50</sub>
	4 %	8 %	12 %	16 %	20 %	
Raw	13.84±0.79 <sup>aA</sup>	42.41±1.14 <sup>bA</sup>	44.00±1.34 <sup>bA</sup>	70.22±2.95 <sup>cA</sup>	77.81±3.21 <sup>cA</sup>	10.72
WBB	47.89±1.11 <sup>aC</sup>	72.77±2.63 <sup>bC</sup>	87.92±1.33 <sup>cC</sup>	88.23±1.92 <sup>cB</sup>	89.21±0.62 <sup>cB</sup>	4.03
MOB	39.30±1.00 <sup>aB</sup>	60.54±1.11 <sup>bB</sup>	75.22±0.70 <sup>cB</sup>	90.19±1.09 <sup>dB</sup>	92.73±1.04 <sup>dB</sup>	5.59

  

Dill Extract	Ferric reducing activity (µ mol Fe (II)/g)				
	20%	40%	60%	80%	100%
Raw	47.70±1.96 <sup>aA</sup>	112.87±0.80 <sup>bAB</sup>	143.29±1.02 <sup>cA</sup>	199.70±1.76 <sup>dA</sup>	242.79±0.88 <sup>eAB</sup>
WBB	63.29±3.66 <sup>aB</sup>	121.04±3.06 <sup>bB</sup>	147.62±3.32 <sup>cA</sup>	194.12±5.06 <sup>dA</sup>	228.29±5.78 <sup>eA</sup>
MOB	51.20±1.36 <sup>aA</sup>	100.37±4.52 <sup>bA</sup>	152.20±2.40 <sup>cA</sup>	191.12±1.28 <sup>dA</sup>	254.37±1.25 <sup>eB</sup>

  

Dill Extract	Total phenol content (mg/100 g)				
	20%	40%	60%	80%	100%
Raw	4.50±0.96 <sup>aA</sup>	18.66±1.85 <sup>abB</sup>	42.83±1.48 <sup>bcA</sup>	61.00±1.60 <sup>cA</sup>	90.91±11.21 <sup>dA</sup>
WBB	3.75±0.43 <sup>aA</sup>	11.16±0.76 <sup>aA</sup>	41.91±0.79 <sup>bA</sup>	60.00±1.08 <sup>cA</sup>	91.00±3.19 <sup>dA</sup>
MOB	2.41±0.65 <sup>aA</sup>	6.41±1.55 <sup>aA</sup>	34.83±3.90 <sup>bA</sup>	59.16±3.75 <sup>cA</sup>	105.00±4.69 <sup>dA</sup>

Where IC<sub>50</sub>, concentration (g/L) for a 50 % inhibition; WBB, water bath boiled; MOB, microwave oven boiled. <sup>a-e</sup>mean±standard error (n=3), followed by different superscripts (lower case letters) in the same row indicates significant differences between treatments ( $p<0.05$ ) within the row. <sup>a-c</sup>mean±standard error (n=3), followed by different superscripts (upper case letters) in the same column indicates significant differences between treatments ( $p<0.05$ ) within the column.

Table 2— Pearson's correlation coefficients between Ferric reducing ability and Total phenolic content of different dill extracts

	Ferric reducing ability			Total phenolic content		
	Raw	WBB	MOB	Raw	WBB	MOB
Ferric Reducing ability	1	1	1	0.981*	0.962*	0.972*
Total Phenolic Content	0.981*	0.962*	0.972*	1	1	1

WBB, water bath boiled extract and MOB, microwave oven boiled extract; \* Correlation is significant at the 0.01 level

**Antibacterial activity of dill extracts**

All the three treatments using dill extracts exhibited activity against both Gram-positive and Gram-negative strains of pathogenic and fish spoilage bacteria tested (Table 3). The MOB dill extract showed maximum antibacterial activity evident from the lowest MIC (250 to 500 µL/mL) and MBC (250 to 1000 µL/mL) values. The lowest antibacterial activity was shown by WBB dill extract which exhibited highest MIC (500 to 1000 µL/mL) and MBC (>1000 µL/mL) values. The MIC of dill extract (Table 3) indicated that Gram-positive bacteria were more sensitive to the extract than Gram-negative bacteria.

**Extension of shelf life of mackerel fillets by MOB dill extract**

Since the microwave heating was fast, economical and yielded an extract having highest antioxidant and antibacterial potential, the MOB dill extract was selected for dip treatment of mackerel fillets to check the shelf life extension of fillets during refrigerated storage. The initial microbial quality of all the three treatment samples indicated an acceptable level of 3.26 log cfu/g (Table 4) considering the proposed upper limit for aerobic plate count of 5.70 log cfu/g for fresh fish<sup>26</sup>. It was observed that TPC of all samples increased with the storage time and that the value of the control sample increased in a faster rate

Table 3— Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of dill extracts against pathogenic and spoilage bacteria

Dill Extracts	<i>Pseudomonas aeruginosa</i>	<i>Aeromonas hydrophila</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>	<i>Yersinia enterocolitica</i>	<i>Vibrio cholerae</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Listeria monocytogenes</i>	<i>Bacillus licheniformis</i>	<i>Bacillus cereus</i>	<i>Bacillus pumilus</i>	<i>Micrococcus sp</i>
Minimum Inhibitory Concentration (µl/mL)													
Raw	500	500	500	500	500	500	500	500	500	500	500	500	500
WBB	1000	1000	1000	500	1000	1000	1000	1000	1000	1000	1000	1000	1000
MOB	500	250	250	250	250	500	500	500	250	500	250	250	250
Minimum Bactericidal Concentration (µL/mL)													
Raw	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
WBB	>1000	>1000	>1000	1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
MOB	1000	500	500	500	500	1000	1000	250	500	500	500	500	500

Table 4— Storage characteristics Mackerel fillets dipped in MOB dill extracts compared to control fillets

Treatment	0 Day	3 <sup>rd</sup> Day	6 <sup>th</sup> day	9 <sup>th</sup> day	12 <sup>th</sup> day
TPC (log cfu/g)					
Control	3.26±0.005 <sup>aA</sup>	4.6±0.005 <sup>bC</sup>	6.12±0.005 <sup>cC</sup>	7.42±0.008 <sup>dC</sup>	8.6±0.005 <sup>eC</sup>
10 %	3.26±0.005 <sup>aA</sup>	4.19±0.003 <sup>bB</sup>	5.41±0.003 <sup>cB</sup>	6.28±0.01 <sup>dB</sup>	6.96±0.005 <sup>eB</sup>
20 %	3.26±0.005 <sup>aA</sup>	4.02±0.005 <sup>bA</sup>	5.21±0.008 <sup>cA</sup>	5.96±0.005 <sup>dA</sup>	6.82±0.003 <sup>eA</sup>
TBARS (mg MDA/ kg)					
Control	1.20±0.13 <sup>aA</sup>	4.29±0.02 <sup>bC</sup>	5.92±0.00 <sup>cC</sup>	7.49±0.03 <sup>dC</sup>	9.29±0.03 <sup>eC</sup>
10 %	1.20±0.13 <sup>aA</sup>	2.50±0.05 <sup>bB</sup>	3.31±0.02 <sup>eB</sup>	4.13±0.01 <sup>dB</sup>	4.79±0.01 <sup>eB</sup>
20 %	1.20±0.13 <sup>aA</sup>	1.96±0.00 <sup>bA</sup>	2.63±0.02 <sup>cA</sup>	3.58±0.04 <sup>dA</sup>	4.30±0.03 <sup>eA</sup>
Sensory Score					
Control	9.00±0.00 <sup>aA</sup>	8.1±0.005 <sup>bC</sup>	7.26±0.008 <sup>cC</sup>	6.36±0.003 <sup>dC</sup>	5.38±0.01 <sup>eC</sup>
10 %	9.00±0.00 <sup>aA</sup>	8.52±0.01 <sup>bB</sup>	7.85±0.01 <sup>cB</sup>	7.18±0.01 <sup>dB</sup>	6.43±0.01 <sup>eB</sup>
20 %	9.00±0.00 <sup>aA</sup>	8.63±0.005 <sup>bA</sup>	7.98±0.01 <sup>cA</sup>	7.32±0.003 <sup>dA</sup>	6.51±0.003 <sup>eA</sup>

mean±standard error (n=3), followed by different superscripts (lower case letters) in the same raw indicates significant differences between treatments ( $p<0.05$ ) within the raw. <sup>A-C</sup>mean±standard error (n=3), followed by different superscripts (upper case letters) in the same column indicates significant differences between treatments ( $p<0.05$ ) within the column.

than that of the samples dipped in MOB dill extract. From the initial level of 3.26 log cfu/g, the TPC of control fillets, fillets dipped in 10 % MOB dill extract and fillets dipped in 20 % MOB dill extract were increased to 8.6 log cfu/g, 6.96 log cfu/g and 6.82 log cfu/g, respectively on 12<sup>th</sup> day of storage. While both the fillet samples treated with MOB dill extract showed a total bacterial count of less than 7.00 log cfu/g at 12<sup>th</sup> day of storage, the control fillet samples exhibited a count of 7.42 on 9<sup>th</sup> day of storage itself.

The quality of fillets during storage was also tested by TBARS test. At the beginning of the storage period TBARS values of fish samples were found to be 1.20±0.13 mg MDA/ kg (Table 4). During storage, the TBARS value of all the three fillet samples significantly increased but the rate of increase was significantly less in fillets treated with MOB dill extracts. This indicated a rapid lipid oxidation in control fillets in which TBARS value increased to 9.29±0.03 mg MDA/ kg in 12 days.

The sensory evaluation of fillets during storage was done to evaluate the overall acceptability of mackerel fillets. The overall acceptance score of control and treated mackerel fillets showed a gradual but significant decrease as the storage time increased (Table 4). While the fillets dipped in 10 and 20 % of MOB dill extracts scored an acceptable level of above 6 throughout storage, the control fillets became unacceptable by 9<sup>th</sup> day. The latter showed a noticeable bitterness, off flavour and decomposition by 9<sup>th</sup> day of storage.

## Discussion

The highest DPPH radical scavenging effect demonstrated by MOB dill extract at 20 % concentration can be attributed to the effective discharge of antioxidant molecules into solution under microwave assisted extraction. There is a previous report of significantly high antioxidant activity measured by DPPH radical scavenging activity in onion extracts obtained through microwave assisted extraction which was attributed to the microwave induced disruption of vacuoles and cell walls at cellular level<sup>27</sup>. The FRAP mechanism is totally electron transfer, so in combination with other methods can be very useful in distinguishing dominant mechanisms with different antioxidants<sup>28</sup>. Redox properties, including free radical scavenging, hydrogen donating and singlet oxygen quenching are mainly responsible for the antioxidant activity of phenolic compounds<sup>29</sup>. The linear positive correlation

between ferric reducing ability and total phenolic contents of different dill extracts suggests that the phenolic compounds are the main components which deliver ferric reducing activity of these extracts. These observations suggests that the excellent antioxidant activity of MOB dill extract was due to its high content of total phenolic compounds and the boiling of dill for a short period in microwave oven facilitates the extraction of phenolic compounds into the solution.

The lowest MIC and MBC exhibited by MOB extract demonstrated the potential of microwave oven boiling as a way to produce dill extract having antibacterial activity to minimize the bacterial deterioration of fish. The lowest antibacterial activity of WBB dill extract may be attributed to the loss of volatiles and/or the physical and chemical changes occurred during prolonged heating in water bath. A decrease in antibacterial activity at high temperature has been reported previously in Irish brown seaweeds and ginger<sup>30,31</sup>. The medium antibacterial activity showed by raw dill extract may be due to the gentle extraction method which was not effective in bringing out the antibacterial molecules into the juice. The sensitivity of different bacteria towards dill extract was varying which suggests the difference in permeability of individual bacterial cell towards bioactive constituents of dill. The Gram-positive bacteria were more sensitive to the extract than Gram-negative bacteria.

It evidently showed that the MOB dill extract significantly delayed the rate of microbial spoilage and extended the shelf life of fish fillet by three days during refrigerated storage at a temperature of 4 °C. It was also observed that as the concentration of dill increased from 10 to 20 %, the total bacterial count decreased significantly during storage suggesting increased efficacy of 20 % MOB dill extract. The increase in TBARS value during storage may be attributed to the partial dehydration of fish during refrigeration and to the increased oxidation of unsaturated fatty acids<sup>32</sup>. The TBARS value of good quality material is considered to be not more than 5 mg MDA/ kg<sup>33</sup>. However, the maximum acceptable limit of TBARS value for chilled or iced fish for human consumption is 8 mg MDA/kg<sup>33,34</sup>. The fillets dipped in 10 and 20 % MOB dill extracts displayed TBARS values lesser than 5 mg MDA/kg throughout the storage, indicating their good quality and delayed lipid oxidation compared to the control fillets.

The control fillets exceeded this threshold of 5 mg MDA/kg by 6<sup>th</sup> day and the limit for consumption i.e. 8 mg MDA/kg by 12<sup>th</sup> day. Hence, it is clear that the MOB dill extracts extended the storage quality of fish fillets compared to control samples. Significantly higher oxidative stability exhibited by MOB dill extract of 20 % concentration compared to that of 10 % conc. suggests greater efficacy of former MOB. The higher sensory score displayed by fillets treated with MOB dill extracts could be attributed to the aroma and better appearance of the treated mackerel fillets. It was observed that when the concentration of dill extracts increased from 10 to 20 % the sensory score increased significantly throughout storage.

### Conclusion

It can be concluded that the extract obtained from dill by microwave oven boiling displayed significant antioxidant and antibacterial activities. The highest antioxidant activity of MOB extract could be attributed to the presence of high amount phenolic content in the extract. The extended shelf life of mackerel fillets treated with microwave oven boiled dill extracts revealed its use as a natural preservative to retain the quality of fish fillets during storage. As the oxidative stability, sensory acceptability and the reduction in total bacterial count of mackerel fillets treated with MOB dill extracts of 20 % concentration was significantly higher, it may be suggested that this concentration would be ideal for the dip treatment of fish fillets.

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