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Comparative Preservative Potential of Essential Oils of *Ocimum gratissimum* from Bambili of the North West and Mbonge of the South West Regions of Cameroon on *Scomber scombrus*

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Authors' contributions

This work was carried out in collaboration among all authors. Authors TCA and NPN designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors TCA, EP and DYY managed the analyses of the study. Authors NAE and WAA managed the literature searches. Authors ATNK and TFPV oversaw the research and writing of the article. All authors read and approved the final manuscript.

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ABSTRACT

Essential oils serve as a natural alternative to chemical or synthetic antimicrobials and antioxidants to fight against food borne pathogens or spoilage organisms, inhibiting lipid peroxidation and extending the shelf life of fish and other seafood. This study examines the antibacterial properties

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of essential oils from leaves of *Ocimum gratissimum L*. from two localities of the North West and South West of Cameroon on some pathogenic spoilage gram negative and positive bacteria isolated from mackerel, and their antimicrobial and antioxidant effectiveness on the fish quality during preservation for one month at -18^{o}C. The plant materials were harvested from Bambili, the North West Region of Cameroon and from Mbonge, the South West Region of Cameroon and the essential oils extracted by hydro-distillation using Clevenger-type apparatus. *Escherichia coli, Salmonella typhi* and *Staphylococcus aereus* were isolated from mackerel by culture techniques and their susceptibility to the essential oils determined by well diffusion method. Psychrophilic bacteria and Enterobacteraceae counts were used to evaluate the microbiological quality of the fish during storage. Total volatile basic nitrogen and thiobarbituric acid reactive substance assays were used as indices to assess the biochemical quality of the fish during storage. Antibacterial susceptibility test showed that essential oils of *O. gratissimum* from the North West and South West Regions were active on all the tested microorganisms with different degree.The inhibitory diameters for essential oil from the South West Region were 28.0 mm, 27.2 mm and 26.0 mm while that for essential oil from the Bambili were 24.1 mm, 20.4mm and 21.9 mm for *Staphylococcus aereus*, *Escherichia coli* and *Salmonella typhi* respectively. At the end of storage periods, the values of Psychrotrophs plate counts, total volatile base nitrogen and thiobarbituric acid reactive substances for fish samples treated with essential oil from the Mbonge were 2.71log$_{10}$cfu/g, 12.88 mgN/100g and 0.88 mgMDA/Kg, while that treated with North West essential oil were 3.00 log$_{10}$cfu/g, 16.24 mgN/100g and 1.26 mgMDA/Kg respectively. From the obtained results, essential oil of *O. gratissimum* from the Mbonge was the most effective in preserving Atlantic mackerel.

Keywords: O. gratissimum L; essential oil; fish quality; antimicrobial property; antioxidant property; fish storage.

1. INTRODUCTION

Fish has high protein content and low saturated fat content, which is considered as highly valuable food [1]. Fresh mackerel fish is highly perishable and can easily deteriorate after being captured due to the endogenous enzyme and rapid microbial growth naturally present in fish or from contamination [2]. Even when refrigerated or frozen to extend its shelf life, these processes may not be sufficient to prevent lipid oxidation or bacteria growth. Lipid oxidation and microbial contamination are the main factors that determine food quality loss and shelf-life reduction [3]. Thus, methods of preventing or delaying such processes are highly relevant in fish processing. In some environmental setup, increase in temperature as a result of power failure may also favour the growth and activities of spoilage microorganisms on fish. Frozen storage in combination with chemical antimicrobials and antioxidant preservatives has been used to delay microbial proliferation and oxidative changes in fish [4]. Although chemical preservatives are highly active, they have been reported to have potential toxicological effect [5,6]. Despite their wide use, these are substances capable of triggering adverse reactions including allergic reactions and carcinogenicity [7,8]. Therefore, it became necessary to develop effective antimicrobial and antioxidant agents from plants that could replace the synthetic preservatives. In this respect that medicinal plants and their products including essential oils have explored as possible candidate for food preservation.

Ocimum gratissimum L. is a plant widely known and used for both medicinal and nutritional purposes. Oils from the leaves have been found to possess antibacterial and antifungal activities [9]. Essential oils from *Ocimium gratissimum* possessed antioxidant and antifungal activities that varied according to the collection sites, West (Dschang) and Centre (Yaounde) Regions of Cameroon [10]. The variation of biological (antioxidant) activities of this plant was also noted in an earlier study [11]. Though *O. gratissimum* has been largely used in different places including Cameroon for medicinal purposes, coupled to its biological activities, no work has been done with respect to its antibacterial and antioxidant benefits on mackerel fish. Also, it is of high relevancy to find out the best areas yielding the highest preservative activities of this plant. Thus, this study aimed at investigating the preservative potentials (antibacterial and antioxidant properties) of the essential oils of *O. gratissimum* of the localities of Bambili (North West Region of

Cameroon) and Mbonge (South West Regions of Cameroon) on mackerel.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh leaves of *O. gratissimum* were harvested in Bambili, Tubah subdivision, Mezam division of the North West Region of Cameroon and in Mbonge, Mbonge subdivision, Meme division of the South West Region of Cameroon on August, 2019. The identification of the plant was done in the Cameroon National Herbarium and voucher specimens was deposited under identification number 30241HNC, Letouzey 3642.

2.2 Animal Material and Culture Media

Atlantic mackerel (*Scomber scrombus*) was used in this study to obtained isolated and purified colonies of pathogenic spoilage gram negative and positive bacteria using specific culture media: MacConkey agar for the isolation of *Escherichia coli*, Salmonella-Shigella (SS) Agar for the isolation of *Salmonella typhi*, Mannitol Salt Agar (MSA) for the isolation *staphylococcus aereus***.**

2.3 Extraction of Essential Oil

Extraction of essential oils from *O. gratissimum* was done by hydrodistillation following procedure similar to that reported by Chand et al. [12]. The plant leaves were hydro-distilled for 4 hours using a Clevenger-typed apparatus. The essential oils obtained were dried over anhydrous sodium sulphate and stored in a brown screw cap glass vials at 4^{o}C until time of use. The extraction yield where calculated as a percentage relative to the fresh plant materials.

2.4 Isolation of Pathogenic Spoilage Bacterial from Mackerel

A 0.5 kg fresh mackerel with smooth surface and no blood trace on the body was purchased from a cold store (CONGELCAM) at Mellen-Yaounde, put in an ice filled flask and taken immediately to the laboratory. In the laboratory, the skin surface slime, dirt, head, wing and fins were removed. The processed fish was crushed with 45 mL of sterilized water in a sterile mortar for 2 minutes to obtain a homogenous inoculum. From the inoculum, three 10-fold serial dilutions were made and 1mL of the original inoculum and each dilution by pipetting 1mL into the center of petriplates containing 15 mL of prepared MacConkey medium, Salmonella-Shigella medium and Mannitol salt medium. The petriplates were then incubated at 37°C for 24 hrs in an inverted position in the Laboratory. Morphological distinct colonies of targeted bacteria that grow on each culture media were selected and further purified on a freshly prepared media and preserved for further studies.

2.5 Antibacterial Susceptibility Test

The susceptibility of the microbes to the essential oils was determined by well diffusion method according to the Clinical & Laboratory Standards Institute recommendations [13]. The susceptibility of the microbes to the two extracts were visually evaluated as inhibition zones surrounding the wells and the inhibitory diameter measured.

2.6 Assessment of the Antibacterial and Antioxidant Effectiveness of Essential Oils on the Quality of Mackerel during Preservation/Storage of Mackerl

2.6.1 Preparation of fish samples for analysis

A 2 kg fresh frozen mackerel was processed into fillets of equal sizes. The fish was divided in to 36 small portions each weighing 15g and treated as follows: Twelve (12) of the fish fillets served as control. Twelve (12) fillets treated with 2mL of essential oil of *O. gratissimum* from the South West Region of Cameroon and the remaining twelve fillets treated with 2 mL essential oil of *O. gratissimum* from the North West Region. The experimental samples were stored in labouratory at -18^{o}C for four weeks during which samples we removed from each treatment at one week internals for analysis.

2.6.2 Microbiological analysis: Assessment of the antibacterial effectiveness of the essential oils on the Psychrotrophic bacteria and Enterobacteriaceae during storage of mackerel

The effect of the essential oil on the growth of psychrophilic bacteria and enterobacteriaceae on the fish during cold storage was evaluated weekly within a storage period of four weeks at -18^{o}C as described by [14]. After each week of storage, samples were withdrawn and the total numbers of Psychrotrophic bacteria and *Enterobacteriaceae* were determined using Plate Count Agar (PCA) and Violet Red Bile Glucose Agar (VRBGA) respectively according to the *International Commission on Microbiological*

Specifications for Foods [15] and Nordic Committee on Food Analysis [16]. Whole fish flesh was processed to fillets of equal sizes, each weighing 15g and divided in to 36 portions for treatment. Twelve (12) of the fish fillets served as control, twelve (12) were treated with 2 mL of essential oil of *O. gratissimum* from Mbonge, the South West region and the remaining twelve (12) fillets treated with 2 mL essential oil of *O. gratissimum* from Bambili, the North West Region. All samples were stored at -18^{o}C after which microbiological analyses of the fish for psychrophiles and enterobacteriaceae was carried out at seven days (one week) interval of storage, for a total of four (4) weeks storage period. The Psychrophilic plates were incubated at 7^{o}C for 48 hours while the *Enterobacteriaceae* plates were incubated at 35^{o}C for 24 hours. After each incubation period, total bacteria colonies were counted and recorded in colony forming units per gram (cfu/g). These were further converted to $\log_{10}$ base values ($\log_{10}$ cfu/g).

2.6.3 Biochemical analysis: Assessment of the effect of the essential oil on the total volatile basic nitrogen (TVB-N) and thiobarbituric acid reactive substances (TBARS)

Measurements of total volatile basic nitrogen (TVB-N) and thiobarbituric acid reactive substances (TBARS) were determined as described by Goulas and Kontominas, [17]. For TVB-N determination, 10g of samples were collected from each treatment at seven days intervals, blended with 50 mL trichloroacetic acid (10% TCA) for 3 minutes and divided into two equal parts. Each part was centrifuged at 400 rpm for 5 minutes, and the supernatant filtered using Whatman N^{o}1 filter paper to obtain a clear extract. The filtrate was distilled in tubes containing 2 g of MgO, 1 drop of silicon antifoaming agent and 100 ml distilled. The distillate was collated in tubes containing 25 mL of 3% aqueous boric acid solution and 0.04 ml of methyl-red indicator for titration of the volatile bases. The distillate (distilled TVBN) was then titrated against aqueous 0.1N hydrochloric acid (HCl) solution to complete neutralization and the TVBN in mg of nitrogen per 100g of fish sample was then determined using the equation;

$$TVBN\ (mgN|100g) = \frac{(V\ X\ C\ X\ 14)}{weight\ of\ sample}\ X\ 100$$

Where V is the volume of HCl added and it concentration (C), 14 represent the molecular weight of nitrogen.

On the other hand, for measurement of thiobarbituric acid reactive substances (TBARS*)*, 10 g of samples were collected from every treatment at one week intervals seven for four weeks of storage, blended with 30 mL trichloroacetic acid (10% TCA) for 3 minutes and 5 ml of ethanolic solution of butylated hydroxytoluene (BHT, 1 g/l) added to prevent further oxidation. The homogenate was centrifuge at 500 rpm for 20 minutes and filtered using Whatman N^{o} 1 filter paper. 1 mL filtrate were heated at 90^{o}C for 30 minutes in tubes each containing 1mL of 0.02 M TBA and 0.1 mL BHT (1 g/l), cooled to room temperature and the absorbance measured at 532 nm. The concentrations of MDA in the samples were determined using the formular:

$$mg\ MDA/Kg\ of\ fish = \frac{Absorbance\ of\ sample\ x\ Molar\ weight\ of\ MDA\ x\ Volume\ of\ extraction\ x\ Dilution\ of\ extract\ added\ to\ TBA\ x1000}{Weight\ of\ sample\ x\ slope\ of\ standard\ curve\ x1000}$$

2.7 Statistical Analysis

All experiments were carried out in duplicates. Data from the experiments were compiled in an excel sheet and analyzed with SPSS software using Mix measures ANOVA with repetitions. The results were expressed as mean ± standard deviation and were regarded significant at $p < 0.05$. Tukey test was used to compare the differences between means of treatment.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Isolation and purification of pathogenic spoilage bacteria

E. coli (A_2), *S. typhi* (B_2) and *S. aereus* (C_2) were isolated from mackerel using MacConkey medium (A_1) Salmonella-Shigella medium (B_1) and Mannitol salt medium (C_1) respectively, and compared with their respective reference plates labelled A_3, B_3 and C_3. *Escherichia coli* on MacConkey medium grows as circular colonies with pink-red colouration. *Salmonella typhi* on Salmonella-Shigella medium grows as circular colonies with black centers while *Staphylococcus aereus* on mannitol salt medium grows as bright yellow colonies (Fig. 1).

3.1.2 Assessment of the inhibitory effect of essential oils from *O. gratissimum* on bacterial pathogens

The essential oils (SW-EO and NW-EO) were tested for their antibacterial activities against pathogenic food spoilage Gram negative (*Escherichia coli, Salmonella typhi*) and Gram positive (*Staphylococcus aereus*) bacteria and the inhibitory zones measured (Fig. 2). The EOs were active on all tested organisms and imparted zones of inhibitions (mm) at different degree. The inhibitory nature of the SW-EO was more pronounced in Gram positive *S. aereus* (28.0 mm), followed by Gram negative *E. coli* (27.2 mm) and *S. typhi* (26.0 mm). Meanwhile, the inhibitory nature of the NW-EO was more pronounced in Gram positive *S. aereus* (24.1 mm), followed by Gram negative *S. typhi* (21.9 mm) and *E. coli* (20.4 mm) (Table 1).

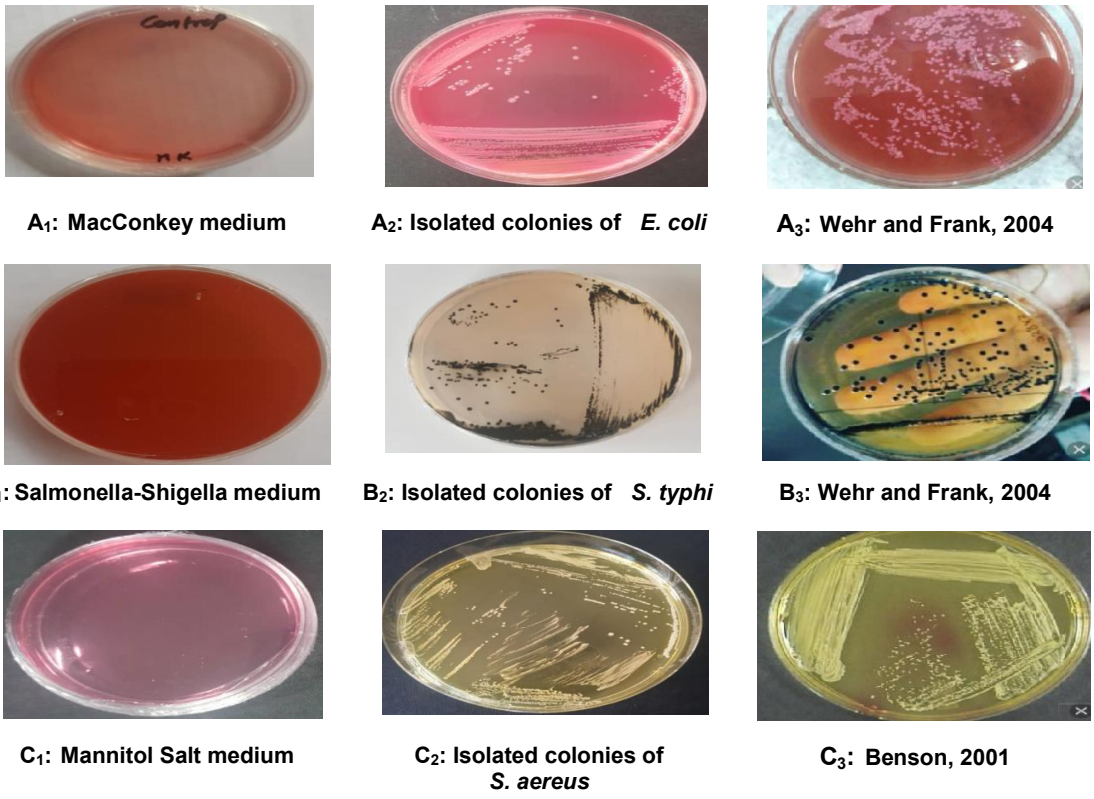

Fig. 1. Isolated pure colonies ({A_1, B_1, C_1 = negative control plates}; {A_2= *Escherichia coli*, B_2 = *Salmonella. typhi,* C_2 = *Staphylococcus aereus*}; {A_3, B_3, C_3 = Reference plates})

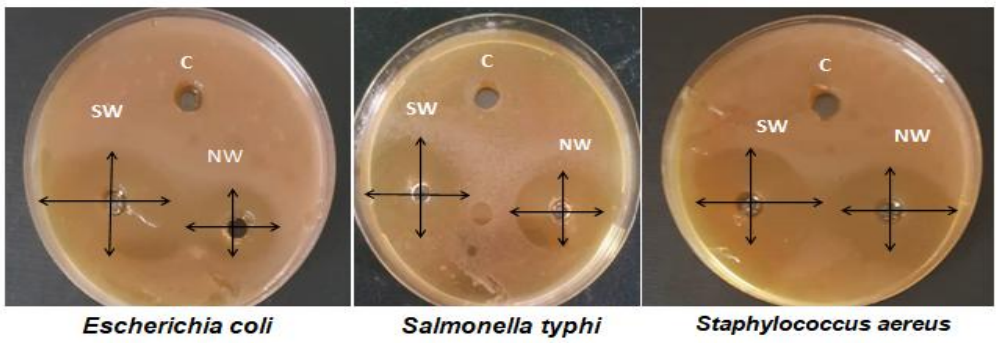

Fig. 2. Plates with zone of inhibition (arrows) of bacteria by the essential oils from *O. gratissimum* harvested in the North West (NW) and South West (SW) Regions of Cameroon

Table 1. Diameter of inhibition (mm) of essential oils on the microbe (Mean ± SD)

Treatments	Diameter of inhibition (mm)		
	E. coli	***S. typhi***	***S. aereus***
C	0.0 ± 0.00^{Aa}	0.0 ± 0.00^{Aa}	0.0 ± 0.00^{Aa}
NW-EO	20.4 ± 0.141^{Ba}	21.9 ± 0.141^{Bb}	24.1 ± 0.282^{Bc}
SW-EO	27.2 ± 0.141^{Ca}	26.0 ± 0.141^{Cb}	28.0 ± 0.141^{Cc}

Means indicated by different capital letters in the same column differ signifcantly (P < 0.05). Means indicated by different lowercase letters in the same row differ signifcantly (P < 0.05). C: control samples, NW-EO: Essential oil from the North West Region, SW-EO: Essential oil from the South West Region

3.1.3 Effect of the essential oils on fish quality during preservation

3.1.3.1-Microbial quality indicators

The number of Psychrotrophs (PBC) and Enterobacteriaceae (EBC) colonies were counted each week and the total number of bacteria per gram calculated. These were further converted to $\log_{10}$ base values ($\log_{10}$ cfu/g). The highest Psychrophilic bacteria count (PBC) was registered for the control sample (4.06 $\log_{10}$CFU/g) during the fourth week of storage and the lowest count for sample treated with SW-EO (2.51 $\log_{10}$CFU/g) during the first week of storage. Similarly, the highest Enterobacteriaceae count (EBC) was recorded for the control sample (3.10 $\log_{10}$CFU/g) during the fourth week of storage and the lowest count for sample treated with SW-EO (2.15 $\log_{10}$CFU/g) during the first week of storage (Table 2).

3.1.3.2 Biochemical quality indicators

3.1.3.2.1 Total volatile basic nitrogen (TVB-N) values of fish treated with and without essential oils of O. gratissimum during storage

The TVBN content in mackerel gradually increase during storage in all the treatments. The TVBN values for the control samples were 12.46, 15.40, 17.92 and 21.28 mgN/100g sample during the first week, second week, third week, and forth week of storage respectively. By using NW-EO, TVBN content were 10.50, 12.88 and 13.86 mgN/100g during the first, second, third and forth week of storage. For SW-EO, TVBN content were 6.30, 9.24, 11.20 and 12.88 mgN/100g during the first, second, third and fourth week of storage respectively. The highest value was observed for the control during the forth week of storage and the lowest was observed for fish sample treated with SW-EO during the first week of storage (Table 3).

Table 2. Bacteria count ($\log_{10}$ cfu/g) of mackerel fish treated with essential oil of *O. gratissimum*

Treatments		Bacteria count ($\log_{10}$ cfu/g) per Week			
		Week 1	**Week 2**	**Week 3**	**Week 4**
Psychrotrophs	C	3.82 ± 0.18^{Aa}	3.95 ± 0.07^{Aa}	4.01 ± 0.11^{Aa}	4.06 ± 0.13^{Aa}
	NW-EO	2.85 ± 0.01^{Ba}	2.93 ± 0.03^{Ba}	2.98 ± 0.04^{Ba}	3.00 ± 0.00^{Ba}
	SW-EO	2.51 ± 0.04^{Ba}	2.55 ± 0.09^{Ca}	2.73 ± 0.04^{Bb}	2.71 ± 0.15^{Bb}
Enterobacteriaceae	C	2.74 ± 0.07^{Aa}	2.88 ± 0.04^{Aa}	2.90 ± 0.00^{Aa}	3.10 ± 0.08^{Aa}
	NW-EO	2.25 ± 0.35^{Ba}	2.47 ± 0.45^{Ba}	2.51 ± 0.39^{Bb}	2.62 ± 0.33^{Bb}
	SW-EO	2.15 ± 0.02^{Ba}	2.30 ± 0.26^{Ba}	2.32 ± 0.23^{Ba}	2.45 ± 0.35^{Ba}

Means indicated by different capital letters in the same column differ signifcantly (P < 0.05). Means indicated by different lowercase letters in the same row differ signifcantly (P < 0.05). C: control samples, NW-EO: Essential oil from the North West Region, SW-EO: Essential oil from the South West Region

Table 3. Total Volitile Basic Nitrogen (mgN/100g) values of mackerel treated with essential oil of *O. gratissimum*

Treatments	Total volatile basic nitogen (mgN/100g) per week			
	Week 1	**Week 2**	**Week 3**	**Week 4**
Control	12.46 ± 1.39^{Aa}	15.40 ± 1.58^{Aa}	17.92 ± 0.40^{Ab}	21.28 ± 1.19^{Ac}
NW-EO	10.50 ± 1.39^{Aa}	12.88 ± 0.79^{Aa}	13.86 ± 0.20^{Ba}	16.24 ± 1.19^{Ba}
SW-EO	6.30 ± 1.00^{Ba}	9.24 ± 0.79^{Ba}	11.20 ± 0.79^{Bb}	12.88 ± 0.79^{Bb}

Means indicated by different capital letters in the same column differ signifcantly (P < 0.05). Means indicated by different lowercase letters in the same row differ signifcantly (P < 0.05). C: control samples, NW-EO: Essential oil from the North West Region, SW-EO: Essential oil from the South West Region

3.1.3.2.2 Thiobarbituric acid reactive substance (TBARS) values of fish treated with and without essential oils of O. gratissimum during storage

The TBARS values for all samples showed increasing trend throughout the storage period. TBARS values for the control samples were 0.68, 0.88, 1.53 and 2.27 mgMDA/kg sample during the first week, second week, third week, and forth week of storage respectively. By using NW-EO, TBARS values were 0.56, 0.60, 0.80 and 1.26 mgMDA/kg during the first, second, third and fourth week of storage. For SW-EO, TBARS values were 0.45, 0.51, 0.62 and 0.88 mgMDA/kg during the first, second, third and fourth week of storage respectively. The highest value was recorded for the control sample during the fourth week of storage and the lowest value for sample treated with SW-EO during the first week of storage (Table 4).

4. DISCUSSION

E. coli, S. aereus and *S. typhi* were isolated from mackerel using MacConkey agar, Salmonella-Shigella Agar and Mannitol Salt Agar respectively. colonies with pink-red colouration were observed on the MacConkey medium. This may be due to the presence of *E. coli* in the fish samples that ferment lactose in the medium, producing acid which change the colour of the neutral red indicator to red and thus grows as red pigmented colonies. A similar colony morphology and colouration was reported by Wehr and Frank, [18] who found that *E. coli* strains cultured on Mac Conkey medium grows as circular colonies with pink-red colouration. On the Salmonella-Shigella medium, we observed circular colonies with black centers. This is possibly due to the presence of lactose non-fermenting *Salmonella typhi* containing thiosuphate reductase enzyme in the fish samples that reduces Sodium thiosulphate present in the medium to H_2S resulting to a black colouration of the colonies [18]. Bright yellow opaque colonies surrounding a yellow medium was observed on the mannitol salt medium. This is possibly due to the presence of *Staphylococcus aereus* in the sample that ferment mannitol, producing acid which lowers the pH of the medium and change the colour of the phenol red indicator in the medium to yellow, resulting to a yellow colouration [19].

The susceptibility test showed that the NW-EO and SW-EO were active on all tested organisms. The antibacterial activity of the essential oils may be due to various groups of potentially active secondary metabolites within them which act by inducing membrane protein and lipid denaturation, inhibition of DNA replication, loss of energy substrate (glucose, ATP), leading to the lysis of the bacteria (cytolysis) and therefore to its death. These findings are in agreement with that in literature documented by Ilori et al. [20] who found that leaf extracts of these plants showed a remarkable activity on *E. coli, S. aereus, S. typhi* and other gram positive and negative bacteria. However, the level of effectiveness of the essential oils from the NW and SW Regions differs. Lemos et al. [21] reported that the antibacterial activities of essential oils of *O. gratissimum* from different Regions in Kenya varies according to geographical distribution of the plants. In this study, the SW-EO showed significantly ($P < 0.05$) larger diameter on all the tested microorganisms than NW-EO. This could be attributed to variation in the chemical composition of the essential oils of the plant from the two regions as a result of differences in soil composition and genetic variability of the plants. The antibacterial activity varied significantly ($P < 0.05$) for the same tested extract within the microorganisms. The Gram-negative *E. coli* and *S. typhi* were generally more resistant to the essential oils than the Gram-positive *S. aereus*. This might be ascribed to the differences in morphological constitutions in the cell wall. Gram-negative bacteria have an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to the essential oils. The Gram-positive bacteria on the other hand are more susceptible, having only an outer peptidoglycan layer which is not an effective permeability barrier [22].

Psychrophilic bacteria count (PBC) and Enterobacteriaceae count (EBC) are routinely used as microbial quality indices. During storage, there was a gradual increase in PBC and EBC in all the treatments. The slight increase could be due to increase in samples nitrogenous compounds (amino acids and nucleotides) and fatty acids produced by hydrolysis of proteins and fats by natural fish enzymes leading to suitable conditions for bacterial growth [23]. Statistically, fish samples treated with NW-EO and SW-EO showed significantly ($P < 0.05$) lower Psychrotrophs and Enterobacteriaceae counts when compared with control samples. A similar effect of clove (*Syzigium aromaticum*) and

Table 4. Thiobarbituric Acid Reactive Substance (mg MDA/Kg) values of mackerel fish treated with essential oil of *O. gratissimum*

Treatments	TBARs (mg MDA/Kg) per week			
	Week 1	Week 2	Week 3	Week 4
C	0.68 ± 0.01^{Aa}	0.88 ± 0.05^{Ab}	1.53 ± 0.216^{Ab}	2.27 ± 0.29^{Ac}
NW-EO	0.56 ± 0.02^{Aa}	0.6 ± 0.0^{Ba}	0.80 ± 0.05^{Ba}	1.26 ± 0.16^{Ba}
SW-EO	0.45 ± 0.04^{Ba}	0.51 ± 0.01^{Ca}	0.62 ± 0.02^{Ca}	0.88 ± 0.05^{Ca}

Means indicated by different capital letters in the same column differ signifcantly (P < 0.05). Means indicated by different lowercase letters in the same row differ signifcantly (P < 0.05). C: control samples, NW-EO: Essential oil from the North West Region, SW-EO: Essential oil from the South West Region

thyme (*Thymus vulgaries*) essential oils on the psychrotrophic bacteria counts (PBC) have been reported by Ozelem et al. [24], who found that leave extracts from these plants decreased significantly the level of psychrotrophic bacteria in tilapia fillets. The ability of these essential oils to decrease the PBC and EBC could result from their hydrophobic nature which enables them to partition in the bacteria cell membranes, disturbing the structure and rendering it more permeable [25]. However, the PBC and EBC for all samples did not exceed the acceptable limit (7logCFU/g) stipulated by ICMSF [15] at the end of the storage period.

The total volatile basic nitrogen (TVB-N) content in mackerel gradually increases throughout the storage period in all the samples. The increase in TVB-N may be the result of deamination of free amino acids, oxidation of amines and degradation of nucleotide by autolytic enzymes and microbial activity [26]. Compared to the control groups, TVB-N levels for samples treated with NW-EO were significantly ($P < 0.05$) lower during the third and fourth weeks of storage while SW-EO treated samples showed significantly lower TVB-N values throughout the storage period. These results indicate that, essential oil from *O. gratissimum* was effective in delaying the rate of formation of TVB-N during storage. This may be attributed to the role of such oil on microbial population and bacterial growth as antimicrobial agents. This activity of essential oil from *O. gratisssimum* is similar to that of essential oil from rosemary plant (*Rosmarinus officinalis* L) reported by Ucak et al. [27], who found that rosemary oil lowered significantly the TVB-N contents in Atlantic mackerel fillets throughout the storage period. At the end of storage, the TVB-N values for all samples did not exceed the acceptability limit (35 mgN/100g) stipulated by EU/EC [28]. To the best of our knowledge very little information is available in literature on the effect of essential oils on TVBN formation in frozen fish.

Thiobarbituric acid reactive substance (TBARS) is an index of lipid oxidation and measures the malondialdehyde (MDA) content and other aldehydes which are secondary products of lipid oxidation [29]. The TBARS content in mackerel gradually increases throughout the storage period in all the samples. The increase in TBARS in all samples with advancing storage time may be due to high fat content in mackerel which favours the continuous lipid hydroperoxide breakdown resulting in production of secondary oxidation products [9]. The difference in the scavenging activity of the essential oils of *O. gratissimum* from the two regions was noticed. Compared with the NW-EO treated samples, the TBARS values for samples treated with SW-EO were significantly lower ($P < 0.5$) during storage. Such findings may be attributed to the high antioxidant e□ect of essential oils of *O. gratissimum* from the South West Regions of Cameroon, which is related to the scavenger nature of their flavonoids and phenolic contents. These results are in line with studies carried out by Fokou et al. [10] who reported that the scavenging nature of essential oils of *O. gratissimum* from the West Region of Cameroon (Dschang) was higher than that from the Centre Region of Cameroon (Yaounde). The antioxidant action of *O. oil* on Atlantic mackerel fillets is also similar to that of rosemary (*Rosmarinus officinalis* L) oil reported by Ucak et al. [27]. At the end of the storage period, the TBARS values in all the frozen samples were lower than the permissible or acceptable limit (4.5mgMDA/Kg) stipulated by EOSQ [30].

5. CONCLUSION

Essential oils have great potential as food preservatives, due to their antioxidant and antimicrobial properties. Taking into account these properties, some essential oils may be considered for applications to different food systems. This research had as main objective to

valorize the essential oils of *O. gratissimum* from Bambili in the North West and Mbonge in the South West Regions of Cameroon by evaluating their preservative potential on mackerel during storage. Isolation and purification of pathogenic spoilage bacteria from mackerel showed that Gram negative *Escherichia coli*, *Salmonella typhi* and Gram positive *Staphyloccocus aereus* were present in the fish sample. Antibacterial susceptibility test showered that the EOs were inhibitory to all tested microorganisms at different degree. The SW-EO was the most active with inhibitory diameter more pronounced in S. aereus followed by E. coli and S. typhi. The PBC, EBC, TVBN and TBARS assays revealed that the essential oils from the two regions preserved mackerel quality by decreasing the level of psychrotrophs, Enterobacteriaceae, total volatile bases and lipid oxidation products respectively during storage, with SW-EO having a higher preserving (antimicrobial and antioxidant) potential.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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