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Antioxidant and Antimicrobial Activities of *Rosmarinus officinalis* L. Growing Naturally in El-Jabal El-Akhdar Province –Libya and its Effect on Keeping Quality of Cold *Serola dumeriri* Fillets

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ABSTRACT

The phytochemical composition, antibacterial and antioxidant activities of methanolic extract produced from leaves of rosemary (*Rosmarinus officinalis* L.) which growing naturally in Libya were assessed. Rosemary extract showed superior scavenging activity in both of Diphenyl-1-picrylhydrazyl (DPPH) (259.67 µmol TE/g dw) and azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) (228.04 µmol TE/g dw). Antimicrobial activity of rosemary against 10 food-borne pathogenic bacteria and food spoilage bacteria were determined and expressed as Minimum inhibitory concentrations (MIC) and Minimum bactericidal concentrations (MBC). Rosemary showed considerable antimicrobial activity against different test bacteria. Namely *Bacillus cereus* and *Enterobacter feacalis* were found to be highly susceptible (MBC: <3.125 mg/ml) whereas *Shigella sonnei* and *Staphylococcus aureus* were the most resistant bacteria (MLC: > 25 mg/ml). Results for phytochemical screening of the methanolic extract of rosemary revealed that total content of phenol and total content of flavonoid were 29.23 mg gallic acid equivalents/gm on a dry basis and 6.59 mg catechin equivalent/g on a dry basis respectively. Additionally, other components were detected consisted of saponin (35.40 mg/gm), tannins (32 mg/gm) and alkaloids (119 mg/gm). Also, rosemary extract (1% aqueous solution) was examined for preservation of *Seriola Dumeriri* fish fillets. Rosemary extract reduced significantly the total viable, psychrotrophic and coliform counts on fish fillets stored at 2°C and extended their shelf life up to 6 days in compared with the untrated samples.

Keywords: Antioxidants, antimicrobial activity, fish, *Seriola Dumeriri* fillets, *Rosmarinus officinalis* L.

INTRODUCTION

The current demand in food industry is to eliminate or reduce the chemical additives from foods. A new approach to protect food from oxidation or prevent the microbial proliferation is the use of natural preservative in foods such as plant extracts or essential oils. Bacterial contamination and lipid oxidation are considered as the main causes of food quality deterioration and shelf-life reduction. Therefore, preventing bacterial contamination of food and delay lipid oxidation are high priority for food processors. Bacterial contamination and lipid oxidation contribute to the deterioration of color, texture and flavour of foods (Fernandez-Lopez *et al*., 2004). The initial loss of fish freshness occur as a result activities of the fish's enzymes and chemical reactions whereas microbial metabolic activities are involved in the whole spoilage. In sea foods, the application of natural and synthetic antioxidants to control lipid oxidation is well established (Khan *et al*., 2006). Researchers are using synthetic antioxidants such as Butyrate hydroxylanisole (BHA), Butylate hydroxyl-toluene (BHT) and Tertiary butyl hydroquinone (TBHQ) to reduce or prevent oxidation problems. Nowadays and due to the carcinogenic and mutating effect of synthetic antioxidants many attempts are being tried to replace natural antioxidants instead of artificial ones (Aubourg *et al*., 2004; Pourashouri *et al*., 2009). To ensure food safety, various studies have been conducted using different preservation strategies to extend the shelf-life of fresh products including fishery products (Sallam, 2007). Preservatives of natural origin, such as *Rosemarinus officinalis* L. has been applied successfully as an antioxidant in many types of fish species (Serdaroglu and Felekoglu 2005;Ibrahim and EL- Sherif, 2008) and Tilapia (Oreochromis niloticus)). In Libya the climate of Mediterranean supports the growth of various number of plant species that have several antioxidant and antimicrobial activities (Naili *et al*., 2010). Rosemary (Rosmarinus officinalis L.) belongs to *Lamiaceae* family is one of the widely spread plant in El-Jabel El-Agadar province and seems to be a rich source of phenolic acids, therefore, it is promising source of natural antimicrobial and antioxidants agents. It is used as flavoring agents in food processing because of its antimicrobial agent, high antioxidant activity and desirable flavor (Ouattara *et al*., 1997 and Lo *et al*., 2002). There are limited data about the application of rosemary for shelf-life extension of fish. To our knowledge there is no detailed study was conducted to reveal antimicrobial or antioxidant potent of Libyan native rosemary Therefore, the aim of the present study was to determine the antioxidant and antimicrobial activities of *Rosmarinus officinalis* growing in El-Jabal El-Akhadar province which is located in east part of Libya. Also to find out the most important active chemical components and to evaluate the effect of rosemary extract on keeping quality of cold *Serola Dumeriri* fillets.

MATERIALS AND METHODS

Plant Materials

Rosmarinus officinalis L. (Rosemary) was collected from fedia region (El-Jabal El-Akhadar province of Libya) in its season (late of spring). The plants were kept in clean plastic bags and transferred to the laboratory where identified authentically and taxonomically in the Horticulture department in faculty of Agriculture, Omer El-Mukhtar University. Plant material was dried in at room temperature (~28°C±1) for 2 weeks after washing with clean. Dried leaves of rosemary were powdered by crushing using an electric blender. Rosemary powder was kept into tightly closed container until extraction.

Methods of Rosemary extract

Extraction of rosemary plant was conducted as described by Ljubuncic, *et al.* (2005). For this, fifty grams of dried rosemary were placed in a conical flask (2000mL capacity) and extracted by adding 500 ml of methanol (80%) with shaking (120 rpm) for 24 hr at room temperature (28°C ±1). Plant debris was removed by

filtration through gauze and the collected extract was filtrated by passing through a filter paper (Whatman no. 1).

The filtrates were lyophilized after concentrating under reduced pressure by rotary evaporator (at 35°C). Yield of rosemary extract was expressed as percentage of the initial amount of plant (50 g) and saved in dark closed bottles at 5°C for further use.

Antibacterial examination:

Potential antibacterial activity of rosemary extract was assessed by using standard bacteria (ATCC: American Type Culture Collection) including food-borne pathogens namely *Aeromonas hydrophila* (ATCC 35654) , *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 19115), *Bacillus cereus* (ATCC 10876) and bacteria usually implicated in food spoilage such as *Pseudomonas fluorescens* (ATCC 49838), *Enterococcus faecalis* (ATCC 19433*), Alcaligenes faecalis* (ATCC 35655) were tested to evaluate the antibacterial properties of rosemary extract. Also, *Salmonella Typhimurium and Escherichia coli* (local clinical isolates; kindly donated by center of biotechnological researches,Tripoli-Libya) were assessed in the present study. Every strains of bacteria were preserved using nutrient agar slants (at 4°C) and reactivated by culturing in nutrient broth for 24 h before testing (Harrigan and McCance, 1966).

Define of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC):

Antibacterial effectiveness of rosemary plant against test food spoilage and food-borne pathogenic bacteria were determined as MIC and MBC. Broth dilution technique as described by National Committee for Clinical Laboratory Standards, NCCLS (1997) (NCCLS) guidelines M7-A4 was used to determine the MIC of rosemary extract. Briefly, the inoculum of each microbial strain was made by growing culture in broth for 12 h and its suspension was prepared to meet 0.5 McFarland standard level. The lyophilized methanolic rosemary extract was dissolved in dimethyl sulfoxide and two-fold dilutions with the range from 1.562 to 25.00 mg/ml were prepared. In separate test tube defined amount of each dilution was added then inoculated with each bacterial suspension and incubated at suitable temperatures for 24 h. After the end of incubation time, the turbidity in test tube indicated growth of the tested bacterium. The MIC value was defined as the lowest concentration of extract exhibited no turbidity and recorded as milligram/milliliter. MBC was determined by spreading loopful taken from each tube on medium of Mueller Hinton agar (MHA, (Oxoid) and incubating the plates at suitable temperatures. MBC was reported as the lowest plant extract concentration which completely inactivated the microorganism (Weerakkody *et al*., 2010). Each test was conducted in triplicate.

Antioxidant capacity of rosemary extract

Scavenging activity of Dipheny l- 1- picrylhydrazyl (DPPH) radical:

Scavenging activity of rosemary methanolic extract using DPPH radicals was assessed by method recommended by of Sudha *et al*., (2012) with slight modification. A mixture consisted of 0.1ml of extract and 1.9 ml of solution of DPPH radical (dissolved in methanol ;0.1 mM) was prepared and after vigorous shaking and setting for one hour in darkness a spectrophotometer (Aquamate Plus UV/Vis; Thermo Scientific, England) was used to measure absorbance of sample at 517 nm. Blank of the test and control was Methanol (80%) whereas Trolox was considered as standard.

Scavenging activity of ABTS radical cation:

Determination of ABTS+ radical scavenging activity of the free radical of rosemary extract was carried out by the spectrophotometric analysis as described by Re *et al*., (1999) and recorded as micromole trolox equivalents per gram of rosemary on the basis of dried weight.

Reducing power of rosemary:

Reducing power of rosemary extract was achieved as described by Vamanu and Nita (2013). In this test different concentrations of (butylated hydroxyanisole) BHA were prepared as a standard and the findings were recorded as milligram of BHA equivalent (BHAE) per gram of rosemary on the basis of dried weight.

Chemical assay of rosemary extract

Evaluation of the total phenolic compound (TPC):

TPC of rosemary extract was determined by folin–ciocalteu method which was described by Singleton and Rossi (1965). Content of Phenolic compounds in the rosemary was recorded as milligram gallic acid equivalents (GAE) per gram of rosemary on a dried basis.

Total flavonoids Content Determination:

Total flavonoids content (TFC) was conducted as described by Yoo *et al*., (2008) which is colorimetric base method. (+)-catechin was used to prepare the standard curve and flavonoid's content was recorded as milligram (+)-catechin equivalent (CE) per gram of rosemary extract on the basis of dried weight.

Saponin content of rosemary extract

Saponins content of rosemary extract was carried out by the method of Obadoni and Ochuko (2002). The saponin amount was recorded as % of the starting weight of rosemary.

Tannins content of rosemary extract:

Vanillin –HCl method was applied to obtain the content of tannin in rosemary as recommended by price *et al*., (1978). After 20 min of incubation at room temperature the absorbance of sample was read at 500 nm. The findings were reported as mg catechin equivalent (CE) per gram of rosemary on the basis of dried weight.

Alkaloids content of rosemary extract

Content of alkaloids was obtained by the method stated by Obadoni and Ochuko (2002).

Fish Sample preparation:

Fresh *Serola Dumeriri* were caught from the coast of Haniya region, El Jabal El-Aghdar-Libya and delivered in isothermal icebox to the laboratory at University of Omer El-Mukhtar, 3 hrs after catching. *Serola Dumeriri* fish was high quality, with a length of up to 1.25 meters, grayish-blue in color, a yellow line along the lateral nerve line, a streamlined body and a crescent tail. It abounds in the Libyan coasts during spring and autumn. Fish guts have been carefully removed, and fish muscle washed and filleted (each fillet was 130 ±10 g). The fillets were dipped into solutions containing 1% of lyophilized methanolic extract of rosemary. Control fillet samples were dipped in sterilized distilled water. All fish fillets groups were placed

in sterilized polyethylene bags after draining and refrigerated at 2 ±1C^{o} for 15 days. Samples were randomly drawn at every three days for analysis with three replicates for each treatment.

Microbiological analysis of fish fillets

Three samples were withdrawn periodically from treated and control fish fillet and fish muscle (10 g) of each treatment was aseptically weighed and put into stomacher bag then homogenized with sterilized 1.5% peptone water using Stomacher Blender (LB400,VWR,UK) for 2 min at room temperature. Serial dilutions were prepared and 0.1 ml from each selected dilution was plated out according to the standard methodologies (Gerhardt *et al*., 1994) to determine the total count of aerobic bacteria, psychrophilic bacteria count and coliform count during a specific time of refrigerated storage. Microbial loads were expressed as $\log_{10}$ cfu/g.

Sensory Evaluation

9-point hedonic scale (Peryam and Girardot, 1952) was performed for sensory analyses to evaluate the consumer acceptability of unfried rosemary treated fish fillets. Fish samples from each treatment were introduced to testers after receiving an explanation of the study and they asked to score for color, smell and overall acceptability from 1 to 9 where 1 represented = dislike extremely,5 = neither like nor dislike (midpoint), and 9 = like extremely). Comprehensive sensory scores were obtained by summing scores of separate tested characteristics.

Statistical analysis

Two-way variance analysis was performed to determine the effect of the rosemary on the extension of refrigerated fish. The averages were isolated when there were significant differences using the Duncan test. Data Analysis was performed by using SPSS software (Version 14.0; SPSS, Chicago, IL).

RESULTS AND DISCUSSION

Due to the fact that most active compounds against microorganisms were extracted from plants and specified as organic compounds either saturated or aromatic and they are usually extracted by organic solvents (Nostro, 2007) methanol was used in this study to extract biologically active ingredients (Kim *et al*., 2005 ; Nair *et al*., 2005 and Kruma *et al*., 2008) from rosemary plant. The findings showed that % yield of rosemary methanolic extract was 17%. The efficacy of methanolic extract of rosemary against test microorganisms on the basis of MIC or MBC are presented in Table (1).

Rosemary had remarkable antimicrobial properties against bacteria. *Bacillus cereus* and *Enterobacter feacalis* were the most susceptible (MBC: <3.125) followed by *Listeria monocytogenes* and *Bacillus subtilis* (MBC: < 6.25) then *Alacligens feacalis* and *Aeromonas hydrophila* (MBC: 12.5) whereas *Staphylococcus aureus* was the most resistant bacteria (MBC: > 25). Several studies (Santoyo *et al*., 2005; Rozan and Jersek, 2009; Li *et al*., 2012 ; and Sarabi *et al*., 2017) reported that rosemary had antimicrobial and antioxidant activities. In general, gram negative bacteria are more resistance to extract than gram positive bacteria and this may due to the fact that the cell wall of Gram-negative bacteria has multilayer structure whereas Gram-positive cell wall is with a single layer (Kozłowska *et al*., 2015). The antibacterial effect of rosemary extract on test bacteria may due to the presence of phenolic compounds which act as antioxidants (Puupponen-Pimiä *et al*., 2001). Moreno *et al*., (2006) stated that rosemary contained high amount of phenolic compounds that have high antimicrobial activity against Gram-negative bacteria and Gram-positive. They reported that the presence of carnosic acid and carnosol were responsible for its high antibacterial activity. Plant phenolics and its constituents may inhibit secondary metabolites production or may have lethal effect on bacterial cells. Major site of interaction with a bacterium is the cell well or cytoplasmic membrane; death of the bacterium can be occurred as a results of damage of membrane by physical disruption leading to deforming in the structure and functionality as a result of losing the interior macromolecules of cell (Viji *et al*., 2017).

Table 1. Antibacterial effect of *Rosmarinus officinalis* (methanolic extract) on test bacteria

Bacterial species	MIC	MBC
	mg/ml	
Escherichia coli	25	25
Salmonella typhimurium	25	25
Aeromonas hydrophila	6.25	12.5
Pseudomonas flurosences	25	25
Alacligens feacalis	6.25	12.5
Enterobacter feacalis	<3.125	< 3.125
Listeria monocytogenes	< 6.25	< 6.25
Staphylococcus aureus	25	>25
Bacillus cereus	<3.125	< 3.125
Bacillus subtilis	< 6.25	< 6.25

MIC: Minimum Inhibitory Concentration.
MLC: Minimum Bactericidal Concentration

Antioxidant capacity:

The antioxidant activity of rosemary extract is returned to the presence of many contents from different categories. It is difficult to identify every single component participate in the antioxidant effect as it takes much efforts and long time. Thus, several methods are applied to determine the capacity of whole extract antioxidant.

Scavenging activities of 2, 2- d ipheny l- 1-picrylhydrazyl (DPPH) and ABTS radical

It is reported that the antioxidant activity of phenolic compounds returns to the effects of their radical scavenging. Scavenging efficacy of radical is very crucial because of the harmful effect of free radicals on biotic systems. In general donation of electrons or hydrogen atom transfer proceeds this process (Niki and Noguchi, 2000). In this study, free radical scavenging activity of *Rosmarinus officinalis* L. methanolic extract was determine by two types of radical activities namely DPPH and ABTS were used. As shown in Table (2) the scavenging effect of DPPH (259.67 ± 0.48 micromol TE/gram of dried weight) and ABTS (228.04 ± 0.59 micromol TE/gram of dried weight) was observed. However, the two types of radical scavenging activity assays showed a similar tendency. Numerous studies indicated that strong antioxidant properties of rosemary extracts (Ozcan, 2003; Gimenez *et al.* 2004; Moreno *et al*., 2006; Cadun *et al*. 2008; Tironi *et al*., 2010). It is reported that certain

compounds in rosemary such as rosmaridiphenol, rosmanol rosmariquinone, and carnosol may have antioxidant activity up to four times equal to BHA and considered as effective as butylated hydroxyltoluene in terms of antioxidant activity (Cadun *et al*. 2008; Rohlík *et al*., 2013).

Table 2. Antioxidant activities of methanolic extract of *Rosmarinus officinalis*

Reducing power (mg BHAE/g dw)	51.98 ± 0.104
ABTS (µmol TE/gdw)	228.04 ± 0.59
DPPH (µmol TE/g dw)	259.67 ± 0.48

±: represent mean ± SD of three replicates.
TE :Trolox Equivalents

Phytochemical analysis of rosemary:

It is well known that the secondary metabolites of plants such as phenolic and/or flavonoids, alkaloids, saponins and tannins exhibit antibacterial and antioxidant effects.Total Phenolic Compounds (TPC) ,Total Flavoniods Compounds (TFC) and others components of rosemary was evaluated and listed in Table (3). Five major class of secondary metabolites were reported: TPC and TFC were 29.23 ± 0.067 and 6.59 ± 0.011 mg of GAE/g respectively. Findings showed that quantity of phenolic compounds were higher in rosemary compared with those of flavonoids and these findings agreed with those obtained by Shan *et al*., (2005) and Wojdylo *et al*., (2007). Phenolic compounds are known to posses components with antioxidative effects that take part in the antioxidant properties of plants (Pezeshk, 2015). In present study, another three major class of secondary metabolites were found in rosemary namely, alkaloids (119 ± 1.6.0 mg/g) , saponins (32 ± 0.006 mg/gm) and tannins (35.40 ± 1.07 mg/gm) (Table 3).

Table 3. Phytochemical analysis of the methanolic extract of Rosemary

Total phenolic compounds (mg *GAE/g dw)	29.23 ± 0.067
Total flavonoids (mg •CE/gdw)	6.59 ± 0.011
Alkaloids (mg/g)	119 ± 1.6.0
Saponins (mg/g)	32 ± 0.006
Tannins (mg/g)	35.40 ± 1.07

***GAE: Gallic Acid Equivalent**
• CE :Catechian Equivalent
±: represent mean ± SD of three replicates.

It is more likely that antioxidant and antimicrobial activity of rosemary due to the existence of some constituents such as phenols, flavonoids and tannins in rosemary (Polterait, 1997 and Hasanuzzaman *et al*., 2013). Tannins are known for their antimicrobial effect (Chung *et al*.,1998; and Hasanuzzaman *et al*., 2013) and antioxidant (Sasaki *et al*., 1989; Hasanuzzaman *et al*., 2013) activities. However, saponins were also reported to inhibit fungal growth (Aboaba *et al*., 2006). It is well reported that the synergistic effect of the existence of various types of chemical components in plant extracts may enhance antimicrobial activity (Parekh and Chanda, 2007).

Flavonoids, alkaloids and tannins extracted from some plants were reported to have antimicrobial activities in *vitro* (Khan *et al*., 2011 and Srinivasan *et al*.,2001). Other researchers found the same phytochemical components in rosemary (Shahlaa *et al*., 2015 and De Almeida *et al*., 2016). However, comparing the polyphenol contents of rosemary tested in present study with those reported in the literature is problematic due to the difference in applied method of analysis, different plant cultivars, part used for analysis and stage of plant maturity (Majhenič *et al*., 2007 and Gouveia-Figueira *et al*., 2014).

Effect of rosemary on shelf life of refrigerated fish fillets storage at 2°Cfor 15 days:

Figure (1) illustrates the total viable counts (TVC) of fish fillets during the period of storage (15-day) at 2°C ±1. As shown in this table by dipping fish fillets into 1%(w/v) aqueous solution of rosemary extract ($p< 0.05$) reduced the bacterial count significantly on fillets samples. The initial TVC of control samples was 5.97 log CFU/g and rosemary treatment exhibited an immediate effect on treated fillets as bacterial count dropped to 4.14 log CFU/g. After fish catching contamination of muscle may quickly occur by some sources such as surface or intestinal bacteria, equipments, aquatic environment, handling or by conditions of storage. In this study, a gradual increase on TVC values of control and treated samples was observed during storage at 2C° starting from day 6. Untreated fish fillets reached a TVC value of 6.4 log CFU/g on the day 3 and still however close to the limit of microbiological acceptability of fresh fish (7) log CFU/g. These results are similar to Ojagh *et al*., (2010) findings who reported that a control fish fillets showed a shelf-life up to 3 days. In present study, a reduction of about 2.6 log CFU/gm in TVC count on treated fillets at day 6 was reported.

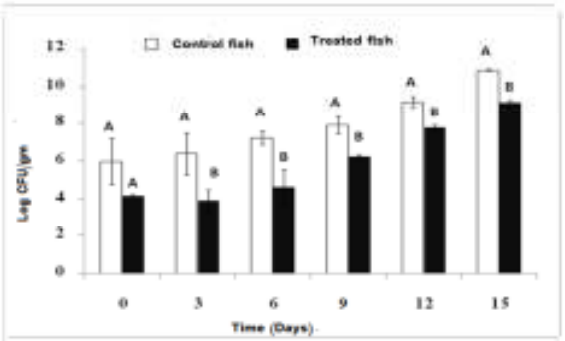

Figure 1. Total viable count of fish fillets treated with rosemary extract during cold storage at 2C° for 15 days. Bars: represent means ± SD of three replicates. For each day significant differences (P <0.05) indicated by different letters on bars.

Treatment led to a significant delay in the microbial growth and caused an extension on the shelf-life of treated fillets until the day 9. Rosemary treated fillets did not reach the microbiological acceptability limit after day 9 of refrigerated storage although significant ($p< 0.05$) lower TVC values were reported compared with those of control samples during the rest of storage time. Similarly, the same pattern of rosemary effect has been observed for psychrotrophic bacteria count (Figure 2) on fish fillets. It evidently showed that rosemary extract delayed the rate of microbial spoilage and increased the shelf life of treated fillets 6 days (until day 9) during refrigerated storage at 2°C for 15 days. Kenar *et al*., (2010) reported that vacuum packed sardine fillets shelf life has prolonged to seven days after dipping fillets in ethanolic extracts of rosemary and stored at 3±1°C. Rosemary extract at 0.4 and 0.8% was

found to be effective in controlling bacterial growth and biochemical indices in vacuum packed Atlantic mackerel burgers during chilled storage (Ucak *et al*., 2011).

Recently, a study carried out by Gao *et al*., (2014) displayed the synergistic effect of rosemary extract along with nisin in inhibiting protein decomposition, lipid oxidation, nucleotide breakdown and microbial growth in pompano fillet (Trachinotus ovatus) throughout the storage at 4°C. Rosemary are reported to inhibit lipid oxidation and microbial growth in several food systems (Li *et al*., 2006 and Zhang *et al*.,2010). Rosemary extract displayed superior antioxidant activity and slowed down lipid oxidation in brined anchovies stored at 4°C for twenty eight days (Turhan *et al*., 2009). Del Campo *et al.,* (2000) reported that at low temperatures, the inhibitory effect of the rosemary extract was enhanced.

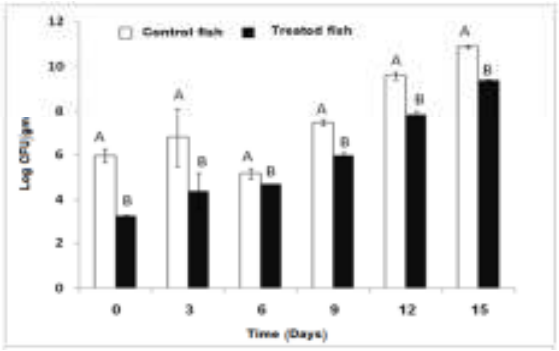

Figure 2. Total psychrophic bacteria count on fish fillets treated with rosemary extract during storage at 2 °C for 15 days. Bars: represent means ± SD of three replicates. In each day, significant differences indicated (*P* <0.05) by different letters on bars.

The coliform bacteria (Figure 3) showed fluctuated pattern for both treated and untreated fish fillets and no significant differences were observed during 15 days of refrigerated storage. It is well documented that rosemary exhibited antibacterial and antioxidant properties (Georgantelis *et al*., 2007). Rosemary extract at 0.2% significantly declined TVC count on air-packaged crucian carp(Carassius auratus) when samples were refrigerated at 4°C for 20 days (Li *et al*., 2012).

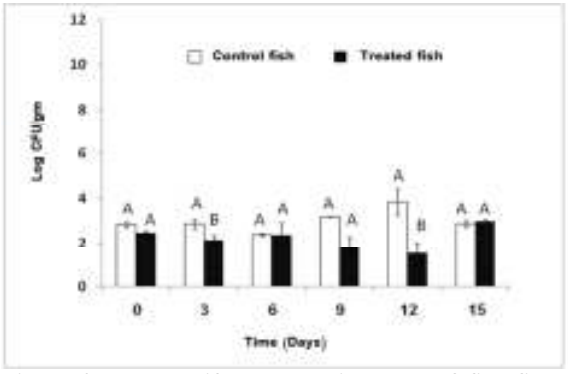

Figure 3. Total coliform bacteria count of fish fillets treated with rosemary extract. Bars: represent means ± SD of three replicates. For each day significant differences (*P* <0.05) indicated by different letters on bars.

The effectiveness of rosemary extract on organoleptic properties of fish fillets

The effectiveness of rosemary extract on organoleptic properties fish fillets was evaluated. Organoleptic properties is a popular test to assess fish freshness as it is fast, simple and can be clearly visible by the consumer (Pezeshk *et al*., 2015). The organoleptic properties impact would be very important if essential oils or plant extracts are widely used as antioxidants or antibacterial agents in marine products. Table (4) illustrates the sensory evaluation findings of fish fillets treated with rosemary and stored at 2C°. No significant differences were observed in all sensory properties between treated and non-treated fish fillets from zero to 3 days of storage period. However, a gradual decrease in all sensory properties and significant differences between the treated fish fillet and control one in the characteristics of smell, colour and overall acceptability were observed from day 6 onwards. By the end of cold storage (Day 15) sensory characteristics of the untreated fish samples reached to unacceptable degree compared with treated samples which were more acceptable.

Table 4. Effect of rosemary extract on organoleptic properties of stored fish fillets

Storage time (days) at 2°C		Smell	Colour	Overall acebtibility
0	Control	8.8[a]	8.7[a]	8.6[a]
	1% rosemary	8.9[a]	8.8[a]	8.7[a]
3	Control	7.5[a]	8.0[a]	7.8[a]
	1% rosemary	8.8[a]	8.9[a]	8.6[a]
6	Control	6.2[a]	6.9[a]	6.1[a]
	1% rosemary	8.0[b]	8.4[b]	8.0[b]
9	Control	4.8[a]	3.7[a]	5.5[a]
	1% rosemary	6.9[b]	6.9[b]	6.5[b]
12	Control	3.0[a]	2.5[a]	3.1[a]
	1% rosemary	6.4[b]	6.7[b]	5.2[b]
15	Control	2.0[a]	2.1[a]	2.2[a]
	1% rosemary	3.2[b]	3.9[b]	4.3[b]

a,b,c for each sensory attribute; in the same day, averages with the same letters have no significant differences at (*P*<0.05).

CONCLUSION

Metabolic extract of rosemary exhibited a good antibacterial effect against tested species of bacteria and its effect was dependent on the species of bacteria. Results indicated that rosemary had a strong antioxidant effect. Treatment of fish fillets with 1% rosemary extract could be effective on delay bacterial growth, retard chemical deterioration, keep the sensory properties and prolong the shelf-life for 6 days of storage compared with untreated fish. Rosemary extract can be applied as a safe treatment for keeping quality of cold *Seriola Dumeriri* fillets.

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النشاط المضادة للأكسدة والمضاد للميكروبات لمستخلص نبات اكليل الجبل *Rosmarinus officinalis* L. النامي بريا في منطقة الجبل الأخضر- ليبيا وأثره على جودة شرائح سمك الشولا المبردة

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تم تقييم التركيب الكيميائي والنشاط المضاد للاكسدة والنشاط المضاد للبكتيريا للمستخلص الميثانولي لنبات (*Rosmarinus officinalis* L.)النامى بريا في منطقة الجبل الاخضر – ليبيا. أظهرت النتائج ان مستخلص إكليل الجبل ذو نشاط عالي مضاد للاكسدة. لكلا من DPPH (259.67 ميكرومول تروليكس /جرام من المادة الجافة) وABTS (228.04 ميكرومول تروليكس /جرام من المادة الجافة). تم تحديد ادنى تركيزات مثبطة (MIC) وأدنى تركيزات مميتة (MBC) للتأكد من النشاط المضاد للميكروبات لاكليل الجبل ضد 10 انواع من البكتيريا المسببة للأمراض المنتقلة عن طريق الأغذية والمسببة لفساد الأغذية. أظهرت النتائج ان الاكليل ذو فعالية مضادة للميكروبات التي تم اختبارها. وجد ان بكتيريا *Bacillus cereus* و *Enterobacter feacalis* شديدة الحساسية لمستخلص الاكليل (التركيز الادنى القاتل من اقل 3.125 مللجم/ مل) في حين كانت بكتيريا *Staphylococcus aureus* أكثر البكتيريا مقاومة (التركيز الادنى القاتل اكثر من 25 مللجم/مل). كشف التحليل الكيميائي للمستخلص الميثانولي لإكليل الجبل أن محتوى الفينول الكلي ومحتوى الفلافونويد الكلي كان 29.23 مللجرام مكافىء حمض الجاليك/جم من الوزن الجاف و6.59 مللجرام مكافئ كاتكين/جم من الوزن الجاف)على التوالي. بالإضافة إلى ذلك احتوى مستخلص الاكليل على saponins (35.40 ملجم/ جم) و tannins (35.40 ملجم/ جم) والفلويدات (1.6.0 ملجم/ جم). كما تم دراسة فعالية المستخلص الميثانولي لاكليل الجبل (1٪) (وزن/ حجم مذاب فى الماء) لاطالة مدة صلاحية شرائح سمك *Seriola Dumeriri* المخزن على 2م°. اظهرت النتائج ان العدد الكلي للبكتيريا والبكتيريا المحبة للبرودة والبكتريا القولونية انخفض بشكل معنوي على شرائح السمك المخزن واطال الاكليل من فترة صلاحية السمك لمدة 6 ايام مقارنة بالعينات الغير معاملة.