Evaluation of the chemical composition and in vitro antimicrobial activity of *Rosmarinus officinalis*, *Zataria multiflora*, *Anethum graveolens* and *Eucalyptus globulus* against *Streptococcus iniae*; the cause of zoonotic disease in farmed fish

Roomiani L.[1*], Soltani M.[2], Akhondzadeh Basti A.[3], Mahmoodi A.[1] Taheri; Mirghaed A.[2] and Yadollahi F.[4]

Received: February 2012 Accepted: November 2012

Abstract

There is a growing interest of industry to replace synthetic chemicals by natural products with bioactive properties from plant origin. The purpose of this study was to elucidate the factors affecting antimicrobial effectiveness of essential oils *Rosmarinus officinalis*, *Zataria multiflora*, *Anethum graveolens* and *Eucalyptus globulus* against food spoilage and pathogenic bacteria, *Streptococcus iniae* ; the cause of zoonotic streptococcosis in fish. Food conservation is based on an intermittent search for foods with a high nutritional quality and microbial stability and it has been reached by the control of the growth / survival of spoiling and pathogen foodborne microorganisms. Based on several reports, fish streptococcosis is currently considered as one of the main limiting factors in the aquaculture industry, due to the significant economic losses (annually more than $150 million) that these infections cause in different cultured fresh and seawater fish species worldwide. The sensitivity of *S. iniae* to antibacterial activity of the essential oils was determined using well diffusion assays and paper disc diffusion method. The ranges of minimum inhibitory concentration (MIC) of the oils and extracts were 3.9–250 and 7.8- 500 µg/ml and the ranges of minimum bactericidal concentration (MBC) values for the oils and extracts were found to be in the range of 7.8-250 and 15.6-500 µg/ml, respectively. The essential oils exhibited antibacterial activity against *S. iniae*. The essential oil of rosemary showed the strongest antimicrobial activity.

Keywords: Essential oils (EO), *Rosmarinus officinalis*, *Zataria multiflora*, *Anethum graveolens*, *Eucalyptus globulus*, Antibacterial activity, Minimum inhibitory concentration (MIC)

1- Department of Fisheries, Science and Research Branch, Islamic Azad University, Tehran, Iran
2- Department of Aquatic Animals Health , Faculty of Veterinary Medicine , Tehran University, Tehran, Iran
3- Department of Food Hygiene, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
4- Department of Food Science and Technology, Shahrkord Islamic Azad University Branch, Iran

*Corresponding author's email: l.roomiani @yahoo.com

Introduction

There has been growing interest in the investigation of natural products for the discovery of active compounds with antimicrobial and antioxidant properties that can be applied to the food industry. As consumers are avoiding the consumption of products with synthetic additives or preservatives, natural active compounds could be an alternative to the employ of synthetic chemicals. Such compounds can be used to prolong the storage stability of food, by inhibiting the growth of foodborne spoilage or pathogenic microorganisms and protecting food from oxidative stress damage. Several authors have tested essential oils of aromatic plants to prolong the shelf-life of food (Mahmoud et al., 2006 ; Fernández *et al*., 2009 ; Gómez-Estaca et al., 2010), while others have focused on the antioxidant and antimicrobial properties of plant extracts and essential oils (Atrea et al., 2009; Harzallah et al., 2011). Foodborne diseases are still a major concern in the world. According to the reports regarding foodborne microorganisms outbreak, *Salmonella enteric, Staphylococcus aureus, Bacillus cereus, Streptococcus iniae, Lactococcus* spp. and *Clostridium botulinum* were found in eggs, beef, chicken, fish and dairy products in the USA and the European Union, as well as in developing countries (Ho et al., 2010).

In the past few decades, the pathogen *Streptococcus iniae* has emerged as a major hindrance to aquaculture operations worldwide, causing economic losses measured in hundreds of millions of dollars annually. In addition, *S. iniae* has established itself as a zoonotic risk, especially in areas of the world that preferentially prepare and consume raw fish. To date, at least 25 human causes of invasive streptococcal infection attributed to *S. iniae* have been confirmed in the USA, Canada, China and Taiwan, many of which were in immunocompromised patients; since there is currently no prospective epidemiological surveillance for human *S. iniae* infections, the true number may be much higher (Milani et al ., 2010).

Rosemary, *Rosmarinus officinalis* L. (Labiatae) is an evergreen perennial shrub grown in many parts of the world. It has been reported to possess a number of therapeutic applications in folk medicines in curing or managing of a wide range of diseases. It is well known that the activity of rosemary extracts in medicine and food industry due to the presence of some important antioxidant oil and phenolic components, to prevent oxidative degradation of oil and lipid containing foods. Rosemary has long been recognized as having antioxidant molecules, such as rosmarinic acid, carnasol, rosmaridiphenol and these have found in ethanol-soluble fraction (Bakırel et al., 2008).

Zataria multiflora is a plant belonging to the Laminaceae family that geographically grows only in Iran, Pakistan and Afghanistan. This plant has the local name of Avishan Shirazi (in Iran) and traditional uses such as antiseptic, anesthetic and antispasmodic. This plant is extensively used as a flavor ingredient in a wide variety of food in Iran. The main constituents of the essential oil of this plant are phenolic compounds such as

carvacrol and thymol (Misaghi and Akhondzadeh, 2007).

Anethum graveolens L. (Umbilliferae), known as dill, is an annual herb growing in the Mediterranean region, Europe, central and southern Asia and it is widely cultured in south eastern region of Iran. The plant is used both medicinally and as an aromatic herb and spice and cookery. Dill has been used traditionally for gastrointestinal ailments such as flatulence, indigestion, stomachache colic and to tract intestinal gas (Yazdanparast and Bahramikia, 2008).

Eucalyptus spp. (family Myrtaceae) originated in Australia, but these plants now grow in almost all tropical and subtropical areas, and are cultivated in many other climates. The genus *Eucalyptus* contains about 600 species. Of all the species, *Eucalyptus globulus* is the most widely cultivated in subtropical and Mediterranean regions. As *Eucalyptus* is a fast-growing tree, and is a suitable ingredient for paper manufacture, there has been extensive overseas forest plantation of *Eucalyptus* trees. Much research has been conducted on the medicinal properties of *Eucalyptus* spp. The leaf extract or essential oil from the leaves of *Eucalyptus* spp. has been reported to possess antifungal, antibacterial, mosquito repellent and antioxidant properties (Salari et al., 2006).

This report provides a basis for further exploitation and use of the four plant resources on human health and food safety. The aim of this study was to determine the chemical composition of *R. officinalis*, *Z. multiflora*, *A. graveolens* and *E. globulus* essential oil and to characterize the in vitro antimicrobial activities of extracts and essential oil against *S. iniae*.

Materials and methods

Preparation of essential oil and extracts

R .officinalis, *E. globulus* and *A. graveolens* were collected from suburbs of Karaj city (Alborz Province, Iran) and *Z. multiflora* Boiss. was collected in Fars Province, south of Iran in June 2011 and identified by Institute of Medicinal Plants, Tehran, Iran. Air-dried aerial parts of the *R. officinalis*, *Z. multiflora* and *E. globulus* and seed of *A. graveolens* were subjected to steam distillation for 2 h using Clevenger-type apparatus. The essential oils were analyzed on an Agilent 6890 gas chromatograph interfaced to an Agilent 5973 N mass selective detector (Agilent Technologies, Palo Alto, USA). A vaporization injector operating in the split mode (1:50) at 250 °C was used , into which a fused silica capillary column (30m length × 0.32 mm internal diameter × 0.25µm film thickness; HP-5MS; 5% diphenyl , 95% dimethyl polydimethylsiloxane, Agilent Technologies) was installed. Briefly, the essential oils were collected from the airdried for 3 h using a Clevenger-type apparatus(Hanil Labtech Ltd., Incheon, Korea), and was dried over anhydrous sodium sulfate for 24 h, measured and stored in hermetically sealed dark-glass containers at -4°C until it was tested and analyzed by gas chromatography/mass spectrometry (GC/MS). The data were acquired under the following conditions: initial temperature 50 °C; program rate 2.5 °C; final temperature 300 °C and injector

temperature 290 °C. The carrier gas was helium and the split ratio was 0.8 ml/min. For GC–MS detection, an electron ionization system with ionization energy of 70 eV was used.

Thirty grams of the dried and powdered plant materials were extracted with methanol by using Soxhlet apparatus at 60 °C for 12 h. The extracts was filtered and concentrated under vacuum at 40 °C by using a rotary evaporator (Heidolph, Laborota 4000, Schwabach, Germany), yielding a waxy material (2.25 g, 7.5% w/w). This extract was suspended in water and extracted with chloroform (4 × 100 ml) to obtain 1.35 g (4.5%) polar and 0.84 (2.8%) non polar extracts. The extract was stored in darkness at 4 °C until used within a maximum period of one week (Jiang et al., 2011).

Bacterial strains and culture conditions

Lyophilized culture of *S.iniae* obtained from Department of Aquatic Animals Health, Faculty of Veterinary Medicine, University of Tehran, Iran, was used in this study. The lyophilized culture was grown in tube containing 10 ml of BHI broth (Merck KGaA, Darmstadt, Germany) twice consequently and incubated each time at 30 °C for 18 h. Then it was followed by streaking on BHI agar (Merck KGaA) slant and incubated at 30 °C for 18 h. The culture was stored at 4 °C as working culture and subcultured at monthly intervals. The bacterial cells of *S. iniae* grown in the broth were adjusted to an optical density (OD) of 0.02 at 600 nm using the Spectronic 20 spectrophotometer (Milton Roy Company, USA). This adjustment gave a cell concentration of 1×10^5 CFU / mL (Moosavy et al., 2008).

Paper disc diffusion method

The disc diffusion method was applied for the determination of antimicrobial activities of the extracts and essential oil (NCCLS, 2000a). Extracts and essential oils were dissolved in dimethyl sulfoxide (DMSO). *S.iniae* were cultured in a nutrient broth (Merck, Germany) for 24 h and diluted with sterilized peptone water. Then, 100 µl of each culture (10^5 CFU) was spread onto the surface of Mueller–Hinton agar (Oxoid, England) to create a bacterial lawn. Sterile blank filter paper discs of 6 mm in diameter (Oxoid, England) were wetted with 20 µl crude extract of *R. officinalis, Z. multiflora*, *E. globulus* and *A. graveolens* (100 mg/ml) and left to dry before being placed on the microbial lawn. The plates were incubated at 37 °C for 24 h. The antibacterial activity was compared with 10 µl of chloramphenicol (1 mg/ml), 20 µl of 5 % lactic acid and 20 µl of 10 % acetic acid. Antimicrobial activity was determined based on the diameter of the clear zone surrounding the paper discs (Celiktas et al., 2007). Three replicate discs were prepared for each extract and essential oil in this study.

Determination of minimal inhibitory concentration

The estimation of the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were measured by the broth microdilution method (NCCLS, 2002). The essential oils were individually dissolved in sterilized physiological saline solution (0.9% w/v) supplemented with Tween 80 (Sigma) at a final concentration of 0.5% (v/v). Serial

doubling dilutions of the oils were prepared in a 96-well microtiter plate in the range 1000 to 7.8 µg/mL. Each essential oil dilution (100 µL) was dispensed into the wells of a microtiter plate; each well was then inoculated with 100 µL of the suspension. The resulting suspensions were mixed with a micro-pipettor. The final concentration of each strain was adjusted to 10^5–10^6 CFU / mL. All microtiter plates against all microorganisms were incubated at 37 °C for 24 h. After incubation, the wells were examined for growth of microorganisms and the MIC was determined. The MIC is defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth. The MBC were confirmed by reinoculating on agar plates with 10 µL of each culture medium from the microplates. The MBC is defined as the lowest concentration of the essential oil at which incubated microorganisms are completely killed. Each experiment was repeated three times (Fu et al., 2007).

Statistical analysis

Differences between extracts and essential oil were tested with analysis of variance (ANOVA). In order to satisfy ANOVA assumptions data were transformed, followed by multiple comparisons tests (Tukey's HSD) to identify differences between groups. All statistical analyses were tested at a 0.05 level of probability with the software STATISTICATM 6.1 (Statsoft, Inc., Tulsa, OK, USA).

Results

Chemical composition of essential oil

Regarding the chemical composition of the essential oils tested, they were shown to be complex mixtures of many components. The relative quantitative values of *R.officinalis* are presented in Table 1. The most important constituents of the rosemary are 1,8-cineole (78.6%) , alpha-pinene (5.87%), toluene (12.26%), Camphor (8.22%) and Berbonone (7.75%). Table 1 shows the identified compounds and percentage obtained by GC/MS, as well as the retention time listed in order of their elution from the DB-5 capillary column.

Table 1: Essential oil composition of *R. officinalis* identified by GC–MS

Compound	Retention time (min)	Percentage
1-Hexanol	8.32	0.35
Alpha-pinene	11.39	15.87
Camphene	12.01	4.20
Verbenene	12.26	0.57
Beta-pinene	13.36	0.48
3-Octanone	13.98	2.76
Beta-myrcene	14.23	2.38
Benzene	15.85	1.15
1,8-cineole	16.24	78.60
Gamma-terpinene	17.60	0.40
1-octanol	18.33	0.39
Linalool	19.80	2.20
Camphor	21.92	8.22
Propanoic acid	22.19	0.82
Berbonone	22.64	7.75

Bicycloheptane	28.69	2.34
Acetic acid	25.27	0.97
Toluene	4.90	12.26
Dodecane	24.80	0.60
Ethanol	8.23	0.04
Naphthalenone	74.33	0.12
Eicosadiene	72.55	1.17
9-Octadecenoic acid	60.24	1.89
Octadecanoic acid	60.76	0.65
Phosphoric acid	68.29	0.47
1,4,7,10,13,16-Hexaoxacycloocta	73.87	2.53
Hexadecanoic acid	54.63	1.39
Alpha- Fenchyl acetate	40.75	1.65
Heptadecene	60.11	2.18
Borneol	23.07	5.45
Butanoic acid	24.36	5.70
15-Nonylphenyl	74.80	0.40
Benzaldehyde	12.64	1.11
1,19-Eicosadiene	72.55	1.17
Gamma-sitosterol	73.95	1.51
Benzaldehyde	12.64	1.11

Volatiles of *A.graveolens* essential oil revealed 21 different compounds accounting for 99.34 % of the essential oil composition that are identified in Table 2.

Table 2: Essential oil composition of *A. graveolens* identified by GC-MS

Compound	Retention index[a]	Percentage
α- Pinene	933	0.31
Sabinene	977	tr[b]
ß-Myrcene	989	0.25
α- Phellandren	1004	0.75
Limonene	1030	19.89
γ-Terpinene	1059	0.34
ρ-Cymene	1090	0.41
Linalool	1096	1.68
E-Limonene Oxid	1139	tr
Estragole	1180	0.94
α- Terpineol	1188	0.17
Z-Dihydrocarvone	1202	6.59
E-Dihydrocarvone	1211	7.36
Cumin aldehyde	1235	0.60
D-Carvone	1243	36.09
Thymol	1294	6.50
Carvacrol	1303	0.21
β- Caryophyllen	1429	0.32
D-Germacrene	1490	0.10
Myristiscin	1527	tr
Dill apiole	1634	16.83

a Retention index on D B - 1 column , [b] tr = trace < 0.1 %

The essential oil of *A. graveolens* contained a complex mixture consisting mainly by D-Carvacrol (36.09%), such as Limonene (19.89%), Dill apiole (16.83%), E-Dihydrocarvone (7.36%) and Z-Dihydrocarvone (6.59%).

Table 3: Essential oil composition of *Z. multiflora* Boiss. identified by GC–MS

Compound	Retention index[a]	Percentage
Thujene	930	0.19
Alpha-pinene	937	4.26
Beta-pinene	976	0.43
Beta-myrcene	985	0.85
Eucaliptol	1024	3.37
Gamma-terpinene	1055	7.34
Linalool	1090	0.68
Thymyl methyl ether	1236	0.47
Carvacrol methyl ether	1243	0.46
Carvacrol	1299	71.12
Trans-caryophyllene	1418	0.41
Globulol	1582	2.32
Sum		91.90

a Retention index on DB5 column .

The yield of the essential oil of air-dried aerial parts of the representative sample of *Z. multiflora* Boiss. was 1.66% (v/w). Percentages of components of the essential oil (as determined by GC–MS) are summarized in Table 3. GC–MS analysis resulted in the identification of 12 components representing 91.9% of the oil. The major compound was phenolic monoterpene carvacrol (71.1%). Other important compounds were gamma-terpinene (7.34%), alphapinene (4.26%) and eucalyptol (3.37%). Table 4 shows, 1,8-eucalypol was the most abundant individual compound in *E. globulus* oil followed by α-pinene (9.22%), α-terpineol acetae (3.1%) and globulol (2.77%).

Table 4: Essential oil chemical constituents of *E. globulus* identified by GC-MS

Compound	Retention time (min)	Percentage
1,8-eucalypol	20.67	72.71
α − terpineol	17.87	2.54
Terpinen-4-ol	19.44	0.34
linalool	24.56	0.24
α − pinene	14.34	9.22
ß-pinene	15.89	0.40
α − eudesmol	26.54	0.39
-globulol	30.74	2.77
epiglobulol	32.89	0.44
α − terpineol acetate	23.21	3.10
geranyl acetate	32.23	0.71
L-pinocarveol	30.24	0.36
ß-sabinen	19.44	0.25
terpinolene	22.19	0.19

Antibacterial activity

The in vitro antibacterial activity of essential oil and extracts of *R. officinalis*, *A. graveolens*, *Z. multiflora* and *E. globules* against *S. iniae* was qualitatively assessed by the presence or absence of inhibition zones. According to the results given in Table 5, extracts of *R.officinalis*, *A. graveolens*, *Z.multiflora* and *E. globules* showed antibacterial effect against *S .iniae* with their respective diameter zones of inhibition of 30, 21, 18 and 16.8 mm whereas the essential oil showed interesting antibacterial effect with inhibition zones in the range of 45-18 mm, respectively. All the tested essential oils showed the antibacterial activity. The best antibacterial activity was observed with the essential oils of *R. officinalis* with larger diameter zones of inhibition, 45 mm (Table 5) and smallest MIC 3.9 µg/mL values against *S. iniae*.

Table 5:Inhibition zone diameters (mm)[a] in well diffusion assays of the *R. officinalis* , *Z .multiflora* , *A. graveolens* and *E. globulus* against *S .iniae*

Plant	***R.officinalis*** Essential oil	extracts	***Z.multiflora*** Essential oil	extracts	***E. globulus*** Essential oil	extracts	***A. graveolens*** Essential oil	extracts
Zone of inhibition	45 ±1.2	30±0.9	22±0.7	18±0.6	18±1.0	16.8±1.3	32±1.1	21±0.8

[a] Each result is the mean ± SD of three replicates.

Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the oils and extracts were determined using a broth microdilution method. The variability in the concentration of the main components present in the essential oils analysed led us to evaluate the antimicrobial activities of the oils and extracts. As shown in Table 6, the MIC values for the oils and extracts of *R. officinalis*, *A. graveolens*, *Z. multiflora* and *E. globules* were found to be in the range of 3.9–250 and 7.8- 500 µg/ml and the MBC values for the oils and extracts were found to be in the range of 7.8-250 and 15.6-500 µg/ml, respectively.

Table 6: Minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) (µg / mL) of essential oil and extracts of *R. officinalis* , *A. graveolens* , *Z. multiflora* and *E. globulus*

	E. globulus				*A. graveolens*			
	Extract		Essential oil		Extract		Essential oil	
S. iniae	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
	500.0	500.0	250.0	250.0	31.2	31.2	7.8	15.6
	R. officinalis				*Z. multiflora*			
	Extract		Essential oil		Extract		Essential oil	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
	7.8	15.6	3.9	7.8	250.0	500.0	62.4	250.0

All tested essential oils are bactericidal and showed MBC values almost equal to MIC values.

Discussion

Although antibiotics have been used to control bacterial infections in fish for many years, several antibiotics have been banned from use in aquaculture due to their adverse effects. In addition, products from aquaculture facilities using antibiotics are not accepted by many countries (Park et al., 2009). Therefore, finding an alternative antimicrobial substance to replace antibiotics has become an issue of interest of many research groups. In cultured fishes, streptococcal infections often develop into lethal septicaemic conditions. Fish in both freshwater and marine environments may be affected, and farms in many parts of the world have consequently suffered serious economic losses. Over the past few years, the Gram-positive bacterium *Streptococcus iniae* has been associated with outbreaks of disease in several species of farmed freshwater and marine fishes (including rainbow trout, tilapia, hybrid striped bass and yellowtail) in the world. The organism has also been isolated from diseased humans. Vaccination was successfully used in rainbow trout farms, but outbreaks resumed thereafter. In particular, such information would be valuable for operation planning, and for the control and prevention of such fish pathogens entering into water column (AL-Bulushi et al., 2010).

Plant-derived essential oils due to their antimicrobial content possess potential significance as naturally occurring agents for food preservation. Many volatile compounds naturally occurring in various essential oils possess strong antibacterial activities, thereby considered as natural antibacterial agents to inhibit the growth of food-borne pathogens. The renewal of interest in food science and technology, and increasing consumer demand for effective natural products means that quantitative data on plant based essential oils is required. The use of essential oils may improve food safety and overall microbial quality. If essential oils were to be more widely applied as antibacterials in foods, the organoleptic impact would be important. On the other hand, volatile oils, which often contain the principal aromatic and flavoring components of herbs and spices, even added into small quantity to foodstuffs, without affecting organoleptic properties, would retard bacterial contamination and therefore reduce the onset of spoilage. For practical addition of essential oils in food, it is important to know if oils at very low concentrations, have efficient antimicrobial effects without affecting sensory qualities (Holley and Patel, 2005).

Essential oils of *R. officinalis, A. graveolens, Z. multiflora* and *E. globulus* have been reported to inhibit a broad spectrum of microorganisms (Ghalem and Mohamed, 2008; Kaur and Arora, 2009; Sarikurkcu *et al*., 2010; Zaouali et al., 2010). The EOs evaluated in this study displayed a variable degree of antimicrobial activity against *S.iniae*. Differences in their chemical profile were observed as well. Regarding this concern, a number of studies on *R. officinalis, A. graveolens, Z. multiflora* and *E. globulus*

EO composition, which reveal a great variability in their chemical profile, have already been published. According to these reports, some components are currently encountered in all species, however in variable amounts (Abed, 2007; Moosavy et al., 2008; Wang et al., 2008; Khan et al., 2009).

As the data of the in vitro antimicrobial activity show, using the filter paper disc agar diffusion technique, the different essential oils inhibited growth to variable extents, depending on the essential oil and the bacterium assayed. Nevertheless, more precise data on the antimicrobial properties of the four studied essential oils were obtained through the determination of the minimum inhibitory concentration and minimum bactericidal concentration. As can be seen in Table 6, the essential oil with the highest antibacterial properties was *R. officinalis* followed by *A. graveolens* while *E. globulus* was the least active essential oil.

The MIC and MBC of essential oils in this study were similar to the known literature with a little difference, which could be for several reasons such as a different growing environment, different extraction methods of essential oils, and so on. Different essential oils have different antimicrobial activity because of their components (Celiktas et al., 2007; Szumny et al., 2010; Ozcan and Arslan, 2011).

The essential oils exhibited antibacterial activity against *S. iniae*. The essential oil of rosemary showed the strongest antimicrobial activity. Also, this study provided comparable quantitative antimicrobial data for four essential oils. The diameter of the zone of inhibition adjacent to essential oils was estimated 45, 32, 22 and 18 mm, respectively (Tabel 5). The extracts of different plant resulted in variable zone of inhibition (30.0–16.8 mm) for *S.iniae*. The results showed that these essential oils can strongly prevent the growth of *S. iniae* GQ 850377.

However, MIC of the *R. officinalis* essential oils and diameter of zone of inhibition in comparison to other essential oils showed potent antimicrobial activity and inhibitory effect. This suggested *R. officinalis* as an effective antimicrobial agent among all kinds of tested herbs.

1,8-cineole acts as a main substance of the rosemary oil. The obtained results are in line with other studies (; Nieto et al., 2010; Zaouali et al., 2010; Jalili-Heravi et al., 2011; Visentín et al., 2011). Also, this activity could be attributed to the presence of oxygenated mono- and sesquiterpenehydrocarbons, and these finding are in agreement with the previous reports (Frutos and Herna´ ndez-Herrero, 2005; AL-Reza et al., 2009; Weerakkody et al., 2010). Among all the essential oils evaluated, *E. globulus* exhibited comparatively weaker antibacterial activity. Such differences can be due to distinct plant origins and essential oil chemical composition. It is generally accepted that essential oils having higher contents of oxygenated terpenes exhibit potent antibacterial potential (Hussain et al., 2011).

The results obtained showed that the essential oils had greater activity, whereas the extracts were found to be less efficient in radical scavenging and lipid peroxidation inhibition (Table 6). In general, extracts exhibited low

antimicrobial activities compared to the essential oils. The oil and the extract, at all concentrations, exhibited higher activities than the control; the differences were significant ($p < 0.01$). But additional investigations need to be performed in order to confirm the safety of these concentrations (MIC) for human consumption. Furthermore, the MBC/MIC ratio is clearly higher than 1, indicating a bacteriostatic effect of the essential oil.

In addition, this study showed that *S. iniae* was more sensitive to essential oil of *R. officinalis* than other essential oils. The MBC of *R. officinalis* was generally lower than the others three oils.

When comparing the data obtained in different studies, most publications provide generalization about whether or not a plant oil or extract possesses activity against Gram-positive and Gram-negative bacteria.

Among all oils analyzed in this work, the essential oil of rosemary was the most effective as an antibacterial agent. The antibacterial activity has been attributed to the presence of some active constituents in the oils. Sensitivity of Gram-positive bacteria to essential oil of rosemary is in line with an earlier study (Oussalah et al., 2006; Kaur and Arora, 2009; Panahi et al., 2011; Teixeira et al., 2012).

Zaouali et al. (2010) showed that *R.officinalis* have more inhibitory effects on Gram - positive bactria than Gram - negative bacteria, which is probably because of the different cell wall membrane structures between Gram-positive and Gram-negative bacteria. Takahashi et al. (2004) showed of the 26 tested bacterial species, the extracts of *E. globulus*, *E. maculate* and *E. viminalis* significantly inhibited the growth of Gram-positive bacteria but these extracts did not show strong antibacterial activities against Gram-negative bacteria.

The essential oil content of different plants varies (together with the biologically active compounds contained) depending on which part of the plant it is obtained from (flower, stem, leaves, whole plant, etc.), the variety of the plant, its harvest season, and the method of cultivation.

These differences might have been derived from local, climatic and seasonal factor. The method of extracting the plant material and its form (whether crushed or finely powdered) affected their antibacterial activity. Essential oils rich in phenolic compounds are widely reported to possess high level of antimicrobial activity which has been confirmed and extended in the present studies. It is believed that the phenolic components of essential oils show strongest antimicrobial activity, followed by aldehyde, ketones and alcohols (Jarrar et al., 2010). However, if plant oils are used for food preservation, issues of safety and toxicity will need to be addressed. Also, it should be undertaken in fish and processed fish to confirm the antimicrobial efficiency level of these essential oils. These essential oils could serve as potential antimicrobial agents to inhibit spoilage growth in food.

Further studies should evaluate the safety and toxicity of *R. officinalis*, *A. graveolens*, *Z. multiflora* and *E. globulus* extracts and essential oils to human consumption before considering their use

for food preservation or medicinal purposes.

Reference

Abed, **K., 2007**. Antimicrobial Activity of Essential Oils of some Medicinal Plants from Saudi Arabia. Saudi *Journal of Biological Sciences*: 14 (1) 53-60.

AL - Bulushi, M., Poole, S., Barlow, R., Deeth, H. and Dykes, G., 2010. Speciation of Gram-positive bacteria in fresh and ambient-stored sub-tropical marine fish. *International Journal of Food Microbiology*. 138: 32–38.

AL-Reza, S., Rahman, A. and Kang, S., 2009. Chemical composition and inhibitory effect of essential oil and organic extracts of *Cestrum nocturnum* L. on food-borne pathogens. *International Journal of Food Science and Technology*. 44, 1176–1182.

Atrea, I., Papavergou, A., Amvrosiadis, I. and Savvaidis, I., 2009 . Combined effect of vacuum-packaging and oregano essential oil on the shelf-life of Mediterranean octopus (*Octopus vulgaris*) from the Aegean Sea stored at 4 °C. *Food Microbiology*. 26: 166–172.

Bakırel, T., Bakırel, U., Keles, O., Gu¨nes, S., Lgen, U. and Yardibi , H., 2008. In vivo assessment of antidiabetic and antioxidant activities of rosemary (*Rosmarinus officinalis*) in alloxan-diabetic rabbits. *Journal of Ethnopharmacology*. 116. 64–73.

Celiktas, O., Kocabas , E., Bedir , E., Sukan , F., Ozek, T. and Baser, K., 2007. Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. *Food Chemistry* 100: 553–559.

Fernández, K., Aspe , E. and Roeckel, M., 2009. Shelf-life extension on fillets of Atlantic Salmon (*Salmo salar*) using natural additives, superchilling and modified atmosphere packaging. *Food Control* 20. 1036–1042.

Frutos, M. and Herna´ndez-Herrero , J., 2005. Effects of rosemary extract (*Rosmarinus officinalis*) on the stability of bread with an oil, garlic and parsley dressing. LWT 38. 651–655.

Fu, Y., Zu , Y., Chen , L., Shi , X,G., Wang , Z., Sun , S. and Efferth , T., 2007. Antimicrobial Activity of Clove and Rosemary Essential Oils Alone and in Combination. PHYTOTHERAPY RESEARCH *Phytotherapy Research* 21, 989–994.

Ghalem , R.B. and Mohamed, B., 2008. Antibacterial activity of leaf essential oils of *Eucalyptus globulus* and *Eucalyptus camaldulensis. African Journal of Pharmacy and Pharmacology*. Vol. 2(10). pp. 211-215.

Gómez-Estaca, J., Lacey, A., López-Caballer , M., Gómez-Guillén , M. and Montero, P., 2010. Biodegradable gelatin-chitosan films incorporated with essential oils as antimicrobial agents for fish preservation. *Food Microbiology*. 27. 889-896.

Harzallah, H., Kouidhi, B., Flamini, G., Bakhrouf, A. and Mahjoub, T., 2011. Chemical composition, antimicrobial potential against

cariogenic bacteria and cytotoxic activity of Tunisian *Nigella sativa* essential oil and thymoquinone. *Food Chemistry*. 129. 1469–1474.

Holley, R. and Patel, D., 2005. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. *Food Microbiology*. 22: 273–292.

Ho, C.H., Noryati, I., Sulaiman, Sh. and Rosma, A., 2010. In vitro antibacterial and antioxidant activities of *Orthosiphon stamineus* Benth. extracts against food-borne bacteria. *Food Chemistry*. 122: 1168–1172.

Hussain, A., Anwar, F., Nigam, P., Sarker , S., Moore, J., Rao, J. and Mazumdar, A., 2011. Antibacterial activity of some Lamiaceae essential oils using resazurin as an indicator of cell growth. LWT - Food Science and Technology 44. 1199 - 1206.

Jalali-Heravi , M., Moazeni , R. and Sereshti , H., 2011 . Analysis of Iranian rosemary essential oil : Application of gas chromatography–mass spectrometry combined with chemometrics. *Journal of Chromatography*. A, 1218. 2569–2576.

Jarrar, N., Abu-Hijleh, A. and Adwan, K., 2010. Antibacterial activity of *Rosmarinus officinalis* L. alone and in combination with cefuroxime against methicillin-resistant *Staphylococcus aureus*. *Asian Pacific Journal of Tropical Medicine* : 121-123.

Jiang, Y., Wu , N., Fu , Y., Wang, W., Luo , M., Zhao , C., Zu, Y. and Liu, X., 2011. Chemical composition and antimicrobial activity of the essential oil of Rosemary. *Environmental toxicology and pharmacology*. 32. 63–68.

Kaur, G. and Arora, D., 2009. Antibacterial and phytochemical screening of *Anethum graveolens* , *Foeniculum vulgare* and *Trachyspermum ammi* . BMC Complementary and Alternative Medicine . doi:10.1186/1472-6882-9-30 .

Khan, R., Islam, B., Akram, M., Shakil, SH., Ahmad, A., Ali , S., Siddiqui , M. and Khan, A., 2009. Antimicrobial Activity of Five Herbal Extracts Against Multi Drug Resistant (MDR) Strains of Bacteria and Fungus of Clinical Origin. Molecules, 14, 586-597; doi: 10.3390/molecules14020586.

Mahmoud, B., Yamazaki, K., Miyashita, K., Shin, I. and Suzuki , T., 2006. A new technology for fish preservation by combined treatment with electrolyzed NaCl solutions and essential oil compounds. *Food Chemistry* 99. 656–662.

Milani, C. J. E., Aziz, R. K., Locke, J. B., Dahesh, S., Nizet , N. and Buchanan, J.T., 2010. The novel polysaccharide deacetylase homologue Pdi contributes to virulence of the aquatic pathogen *Streptococcus iniae*. *Microbiology*: 156, 543–554.

Misaghi, A. and Akhondzadeh Basti, A., 2007. Effects of *Zataria multiflora* Boiss. essential oil and nisin on *Bacillus cereus* ATCC 11778. *Food Control*. 18. 1043–1049.

Moosavy, M. H., Akhondzadeh Basti , A., Misaghi , A., Zahraei Salehi , Z., Abbasifar, R., Ebrahimzadeh

Mousavi, H. A., Alipour, M., Emami Razavi , N., Gandom, H. and Noori, N., 2008. Effect of *Zataria multiflora* Boiss. essential oil and nisin on *Salmonella typhimurium* and *Staphylococcus aureus* in a food model system and on the bacterial cell membranes . *Food Research International*. 41 : 1050–1057.

NCCLS (National Committee for Clinical Laboratory Standards). 2000a . Performance standards for antimicrobial disc susceptibility tests. Approved Standard, M2-A7.

NCCLS (National Committee for Clinical Laboratory Standards). 2002. Performance standards for antimicrobial susceptibility testing. 12th. Informational Supplement, M100-S12.

Nieto, G., Díaz, P., Bañón, S. and Garrido, M., 2010 . Dietary administration of ewe diets with a distillate from rosemary leaves (*Rosmarinus officinalis* L.): Influence on lamb meat quality. Meat Science: 84. 23–29.

Oussalah, M., Caillet, S., Saucier, L. and Lacroix , M., 2006. Antimicrobial effects of selected plant essential oils on the growth of a Pseudomonas putida strain isolated from meat. Meat Science 73: 236–244.

Ozcan, M. and Arslan, D., 2011. Antioxidant effect of essential oils of rosemary, clove and cinnamon on hazelnut and poppy oils. *Food Chemistry* 129: 171–174.

Panahia, Y., Sattarib, M., Babaiec, A., Beiraghdard, F., Ranjbare, R., Hedaiatjoo, A. and Bigdeli, M., 2011. The Essential oils activity of *Eucalyptus polycarpa*, *E. largiflorence*, *E. malliodora* and *E. camaldulensis* on *Staphylococcus aureus*. *Iranian Journal of Pharmaceutical Research*: 10 (1): 43-48.

Salari, M. H., Amine, G., Shirazi , M. H., Hafezi, R. and Mohammadypour, M., 2006. Antibacterial effects of *Eucalyptus globulus* leaf extract on pathogenic bacteria isolated from specimens of patients with respiratory tract disorders. Clinical Microbiology and Infection, Volume 12. Number 2.

Sarikurkcu, C., Ozer , M., Eskici, M., Tepe, B., Can , S. and Mete, E., 2010. Essential oil composition and antioxidant activity of *Thymus longicaulis* C. Presl subsp. longicaulis var. longicaulis. *Food and Chemical Toxicology* 48. 1801–1805.

Szumny, A., Figiel , A., Gutierrez-Ortiz , A. and Carbonell-Barrachina , A., 2010. Composition of rosemary essential oil (*Rosmarinus officinalis*) as affected by drying method. *Journal of Food Engineering*. 97 : 253–260.

Takahashi, T., Kokubo, R. and Sakaino, M., 2004. Antimicrobial activities of eucalyptus leaf extracts and flavonoids from *Eucalyptus maculate*. Letters in Applied Microbiology. 39, 60–64.

Teixeira, B., Marquesa, A., Ramosa, C., I Batista , I., Serranoc, C., Matosd, O., Nenge, N., Nogueirae, J., Saraivab, A. and Nunesa, M., 2012. European pennyroyal (*Mentha pulegium*) from Portugal: Chemical composition of essential oil and

antioxidant and antimicrobial properties of extracts and essential oil. *Industrial Crops and Products*. 36: 81–87.

Visentín, A., Cismondi, M. and Maestri, D., 2011. Supercritical CO2 fractionation of rosemary ethanolic oleoresins as a method to improve carnosic acid recovery. *Innovative Food Science and Emerging Technologies*. 12. 142–145.

Wang, W., Wu, N., Zu, Y. and Fu, Y., 2008. Antioxidative activity of *Rosmarinus officinalis* L. essential oil compared to its main components. *Food Chemistry* 108. 1019–1022.

Weerakkody, N., Caffin, N., Lambert, L., Turnera, M. and Dykes, G., 2010. Synergistic antimicrobial activity of galangal (*Alpinia galanga*), rosemary (*Rosmarinus officinalis*) and lemon iron bark (*Eucalyptus staigerana*) extracts. *Journal of the* Science *of Food andAgriculture* 91: 461–468.

Yazdanparast, R. and Bahramikia , S., 2008. Evaluation of the effect of *Anethum graveolens* L. crude extracts on serum lipids and lipoproteins profiles in hypercholesterolaemic rats. DARU Vol. 16, No. 2.

Zaouali, Y., Bouzaine, T. and Boussaid, M., 2010. Essential oils composition in two *Rosmarinus officinalis* L. varieties and incidence for antimicrobial and antioxidant activities. *Food and Chemical Toxicology* 48: 3144–3152.