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# *In vitro* and *in situ* inhibition of some food-borne pathogens by essential oils from date palm (*Phoenix dactylifera* L.) spathe

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ABSTRACT

Essential oils extracted by hydro-distillation form date palm spathe (byproduct from date palm plants) were tested for their antibacterial activity against some food-borne pathogens. *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 29243, *Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076 and *E. coli* ATCC 25922 were inhibited (11–13 mm inhibition zones) by spathe essential oils (SEOs) using the agar well assay (*in vitro* test). *Staphylococcus aureus* ATCC 29243 and *E. coli* ATCC 25922 were not detected in chicken meat treated with 1% (v/w) SEOs and subjected to abusive storage conditions (20 °C for 18 h). When treated with 0.5% SEO, counts of *S. aureus* and *E. coli* increased by only 0.2 and 0.7 $\log_{10}$ cfu/g, respectively, compared to the initial inoculated level in meat samples stored at 20 °C for 18 h. SEOs possessed DPPH radical scavenging activity with $IC_{50}$ of 0.61 μg/ml. Forty one compounds were major constituents detected by GC–MS analysis of SEOs. 3,4-Dimethoxytoluene (38.12%) and 5,9-Undecadien-2-one (12.45%) were major compounds in extracted oils. Density and refractive index of SEOs were 0.987 and 1.5905, respectively. SOEs are added-value products from date palm, which could be employed in food industry and pharmaceuticals. The study is the first report on antibacterial activity of SEOs against *L. monocytogenes* ATCC 7644 and other standard food-borne pathogens in agar diffusion assay and food model (chicken meat). DPPH radical scavenging activity of SEOs has not previously been documented.

## 1. Introduction

Food spoilage and poisoning resulted from microorganisms lead to food-borne illnesses and economic losses. Food-borne diseases are a public threat affecting human health, social-economic developments and the environment. The implicated agents are bacteria, parasites, viruses, toxins, metals and others. However, the greater risk has been associated with bacterial pathogens because of their emergence, re-emergence nature as well as adaptation to various niches (Bouyahya et al., 2017; Ssemanda et al., 2018). In fact, a recent report indicated that severe *Salmonella* spp. infections were implicated in colon cancer (Ssemanda et al., 2018). *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium* and *Staphylococcus aureus* are the major pathogens in the USA. Annually, the CDC of the USA reported more than sixty one deaths from 73,000 cases of *E. coli* O157:H7 infections. It was estimated that disease cost resulting from pathogenic bacteria was seventy seven billion US dollars per a year (Harich et al., 2018).

Food processing techniques, such as chemical preservatives and antioxidants and cooling could not eliminate food pathogens and delay microbial spoilage as well as inability to retard quality deteriorations (*e.g.*, lipid oxidation) of food. Oxidative deteriorations result in alterations of organoleptic properties of food products which become unacceptable to consumers. Although oxidation is a vital process, it causes formation of reactive oxygen species (ROS), including free radicals (*e.g.*, anion radicals $O^{2-}$) and non-free radical species, such as hydrogen peroxide. Consumers, research communities, the food industry and food legislative bodies have an ever increase interest in using natural antimicrobials and antioxidants in food preservation to inhibit growth of food-borne pathogens and to improve safety and quality of products. Those natural alternatives could be effective in maintaining safety and quality of food products. Essential oils (EOs) are natural extracts from whole plants or their leaves, flowers, buds, seeds, fruits or roots. They are the major economic products obtained from plant distillation techniques (D'Amato et al., 2018). Industrial productions of EOs have been estimated at 50 to 100 tonnes every year. Besides, the price of EOs ranged from 6 to 45 Euro/kg (Baltia et al., 2018). The Association Française de Normalization (AFNOR) and the European Pharmacopoeia (Ph. Eur.) define EOs as pure, identified raw materials of plant origins,

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obtained by hydro-distillation or mechanical processes (Do et al., 2015). The EU, USA, China and other countries have permitted uses of some EOs in food preservation (Ju et al., 2018). EOs and their constituents have been demonstrated to have various biological and pharmacological activities, such as antibacterial and antioxidant properties, among others (Bouyahya et al., 2017; Ud-Daulaa et al., 2016). They have received great attention as sources of new medicines and pure natural ingredients for fragrance, cosmetics and the food industry. Despite the fact that artificial food additives are approved for use in food, there has been an increasing demand for more natural and effective alternatives for the chemical ones. Date fruit palm (*Phoenix dactylifera* L.) is an important plant in dry regions of Arabian Peninsula, central and Southwest Asia, North Africa and other world regions. Palm tree represents an important symbol in Judaism, Christianity and Islam (Plotkin and Balick, 1984). Date palm fruits are commonly used as an important source of nutrition, especially in the arid areas where few plants could grow. Spathes are one of the major wastes of palm trees. The utilization of plant byproducts would have both economic and environmental benefits as a source of inexpensive natural antimicrobials and other phytochemicals (Gyawali and Ibrahim, 2014). Spathe is the leaf structure surrounding pollinating organs of palm trees (Demirci et al., 2013). Spathe hydro-distillates have been used in traditional medicine in the Arabian Peninsula and other Asian countries. Additionally, spathe hydro-distillate is added as a flavoring substance to drinks (tea and water) and other food (Al-zoreky and Al-Taher, 2015). In a previous report, methanol extracts (ME) of fresh spathe were active against *E. coli* ATCC 25922, *Salmonella enterica* ATCC 13076, *L. monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 29213 and other bacteria. However, water or organic solvent (*e.g.*, petroleum ether) extracts of fresh spathe and other plant extracts were inactive against different microorganisms (Al-zoreky, 2009; Al-zoreky and Al-Taher, 2015). Interestingly, ME of dried spathe were either less active or inactive towards pathogens, as compared to the fresh ones (Al-zoreky and Al-Taher, 2015). That finding was tempting to explore some of the biological activities (*i.e.*, antibacterial and antioxidant activities) of volatile constituents in fresh spathe. It was emphasized that shelf life extension investigations (psychrotrophic counts) on fresh chicken meat would not assure product safety. Thus, determination of potential food-borne pathogens in chicken meat is necessary for safety concern (Chouliara et al., 2007). In that sense, spathe essential oils (SEOs) were tested for their *in vitro* (agar diffusion assay) and in a food system for their inhibitory activity against some food-borne pathogens. Some physicochemical properties, DPPH Radical scavenging activity and GC–MS analysis of SEOs were also conducted.

## 2. Materials and methods

### 2.1. Plant samples and preparation

Fresh and healthy spathe samples were obtained from different local farms in Al-Ahsa (Eastern Province, Saudi Arabia) during flowering seasons (February–March 2016 and 2017) of female plants. The moisture of samples was 54.59% ± 2.598, as determined by air drying until constant weight was obtained. Fresh materials were prepared for hydro-distillation by washing with double distilled water (DDW) to remove dirt and extraneous materials. Access water was removed by spreading samples on absorbent papers. Prepared spathe units were cut to small pieces (~2 × 2 cm) using a garden scissor.

### 2.2. Extraction of EOs

Four hundred grams of prepared samples were added to 1 L-capacity flask (round bottom) of Clevenger-type distillation units situated on a four-place heating mantle. Five hundred ml DDW was poured into flask contents and hydro-distillation, at medium heat, was continued for 2 h. Distillation time was counted when hydro-distillates appeared in condensers. From preliminary experiments, no more SEOs were extracted from samples after 2 h distillation period. Sample distillates were collected in clean flasks followed by cooling to room temperatures. Hydro-distillate portions were filter sterilized (0.45 μm membranes) and set aside for later on to do antibacterial testing (Section 2.5.1 below).

SEOs were extracted from hydro-distillates (350 ml portions) with n-hexane (2 × 100 ml) in a separatory funnel. After 1 h under ambient temperatures, the aqueous layer (hydrosols) was carefully removed to clean flasks for further hexane extraction. The organic layers (n-hexane fractions) were collected in amber bottles. Hexane was evaporated *in vacuo* at 30 °C using a rotary evaporator (Eyela N-1200B, Tokyo Rikakikai Co., Japan) equipped with a cooling system. SEOs were subjected to centrifugation at 6000 rpm for 10 min at 4 °C. (Z 326 K Hermle, Germany). Clear SEOs was stored in amber-glass vials and kept refrigerated until further analyses.

### 2.3. Physicochemical properties of SEOs

Yield (%) of SEOs was calculated based on dry weight (dwb) of fresh samples. Density and refractive index of SEOs were measured according to previous methods (Mayo et al., 2011; Ud-Daulaa et al., 2016). Drummond micro-capillaries and an Abbe refractometer (Milton Roy Company, USA) were, respectively, used for density and refractive index determinations of SEO. Means of triplicated experiments were calculated.

### 2.4. GC–MS analysis of SEOs

SEOs constituents were identified using a GC–MS protocol similar to that of Li et al. (2013). A Shimadzu GC/MS-QP 2010 Plus (Shimadzu Corporation, Tokyo, Japan) was used for the chemical composition of extracted oils. The splitless injection mode was set at 250 °C. A DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm) was used. The oven temperature was programmed as follows: from 40 °C (3 min hold) raised at 4 °C/min to 280 °C (20 min hold). Helium was the carrier gas (1.2 ml/min) and MS were recorded at 70 eV, ion-source temperature was 200 °C, and the interface temperature was 280 °C. Five μl of diluted sample (1:50 in methanol) was injected *via* auto-sampler. EOs components were identified by MS comparison with NIST 08S Library (National Institute of Standards and Technology, USA). Relative component amounts were expressed in % area according to peak area in total ion chromatogram (TIC).

### 2.5. Antibacterial activity

#### 2.5.1. Bacterial strains and in vitro assay

Two-gram positive (*Listeria monocytogenes* ATCC 7644 and *Staphylococcus aureus* ATCC 29213) and two gram-negative bacteria [*Escherichia coli* ATCC 25922 and *Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076 (*Salmonella enterica* ATCC 13076)] were used in the study. Bacteria were tested for their sensitivity to SEOs using an agar well diffusion assay. Test pathogens were maintained and prepared as previously described (Al-zoreky and Al-Taher, 2015). Stock cultures were maintained at −20 °C in BHI containing 10% sterile glycerol. Working cultures were activated in nutrient broth at 37 °C for 18 h. Bacterial counts were confirmed by plating out in Nutrient agar (NA, Oxoid, UK) and incubation at 37 °C for 48 h. Ten μl (*ca.* $10^8$ colony forming unit (cfu)/ml, except *L. monocytogenes* ATCC 7644 *ca.* $10^7$ cfu/ml) active culture was aseptically added to a test tube containing 8 ml of sterilized and tempered (water bath at 50 °C) NA. The mixture was immediately vortexed to evenly distribute inoculum in agar followed by pouring into plastic Petri plates (sterile, Ø 90 mm). After solidification, wells (6 mm diameter) were made using a sterile stainless-steel borer. To each well, 20 μl of SEOs or hydrosols (filter sterilized, 0.45 μm membrane filters) were added. The reference was

Pristinamycin IA (20 μg/ml acetonitrile). Acetonitrile was the blank which had no inhibition for test pathogens. Plates were left undisturbed for 30 min at ambient conditions to allow diffusion of test materials. Plates were incubated at 37 °C for 12–48 h, until visible growth of bacteria was evident in control plates. Diameters in mm of inhibition zones (including well diameter) were measured. The *in vitro* test was done in triplicates.

#### 2.5.2. In situ antibacterial activity

It was reported plant extracts, such as EOs had less or no activity in food matrices (*e.g.*, meat) compared to their performance in *in vitro* tests (Hao et al., 1998; Burt, 2004; Jayasena and Jo, 2014; Stojanovic-Radic et al., 2018). Thus, it was appropriate to evaluate the antibacterial activity of SEOs directly in a food system. One gram-positive and –negative bacteria were chosen, based on their sensitivity to SEOs in the above screening assay (agar diffusion). The inhibition activity of SEOs was done using a food model (chicken breasts). *Staphylococcus aureus* ATCC 29213 and *E. coli* ATCC 25922 were chosen for challenge experiments. Fresh chilled chickens were obtained, within their shelf-life dates, from some outlets of poultry brand name processing plants. Under aseptic conditions, plastic packages of samples were discarded followed by skin removal from whole chickens. Excess fats were trimmed and raw meat was cut into small and thin pieces (2 g, ~1.5 × 1.5 × 0.5 cm). Samples were decontaminated in boiling DDW for 10 min in glass beakers. During thermal treatments, beakers were tightly covered with two layers of aluminum foils to maintain proper temperatures. Meat pieces were aseptically transferred to sterile petri plates and excess water was removed by gentle pressing with sterile forceps. Pieces were dried for 10 min in a laminar flow hood followed by UV treatments for 30 min. A portion of boiled and UV treated samples were tested for sterility by plating in NA followed by incubation at 37 °C for 48 h. They had below detection limit counts (< 1 $\log_{10}$ cfu/g). Ten μl of active culture of *Staphylococcus aureus* ATCC 29213 or *E. coli* ATCC 25922 was evenly spread on one side of each piece (3 $\log_{10}$ cfu/g). Inoculated samples were kept refrigerated for approximately 18 h to allow adherence of bacterial cells. SEOs were then evenly spread on inoculated sides of chicken pieces using 20 μl methanol as a dispersing solvent. Final concentrations of SEOs in samples were 0.125, 0.5 and 1%. Controls were treated with methanol. For comparison purposes, α-terpineol (1%) was used. Spreading EOs on food samples was chosen to control amounts applied and quantities absorbed of test materials into food matrices, as opposed to dipping techniques. Control and treated samples were maintained in covered and sterile petri plates. Plates were kept under abusive storage conditions (20 °C for 18 h) in an incubator (LabTech, Republic of Korea). In this regard, Stojanovic-Radic et al. (2018) indicated that meat storage at 4 °C was below lower limit for growth of bacteria in controls. Thus, it was difficult to assess antibacterial activities of EOs in chicken meat (Stojanovic-Radic et al., 2018). After storage at 20 °C for 18 h, test pathogens were enumerated in samples diluted in sterile 0.1% Peptone-NaCl (Oxoid, UK) water and vortexed to dislodge bacterial cells. 100 μl was pipetted on NA plates and spread using a sterile glass rod. Plates were incubated at 35 °C for 48 h prior to colony counting. The experiment was conducted in triplicates.

### 2.6. Antioxidant activity of SEOs

The DPPH radical scavenging activity protocol (Gourine et al., 2010) was used with a modification (ethanol was replaced by methanol for DPPH solution preparation). DPPH assay is the most widely used *in vitro* test used to assess free radical scavenger capacity of potential antioxidants (Dussault et al., 2014). The violet color of DPPH solution, with a strong absorbance at 517 nm, decolorized in a stoichiometric manner depending on hydrogen donating ability of antioxidants (Gourine et al., 2010). Different concentrations (0, 3.125, 6.25, 12.5, 25, and 50 μg/ml methanol) of SEOs were prepared. Fifty μl of prepared concentrations was added to 950 μl of 100 μM DPPH solution. Final concentrations of SEOs in reaction mixtures were 0.16, 0.31, 0.63, 1.25 and 2.5 μg/ml. Reaction cuvettes were left in the dark at ambient temperatures for 30 min. Absorbance (A) values of samples against blank (50 μl of methanol in DPPH solution) at 517 nm were recorded (Evolution 201 spectrophotometer, Thermo Scientific, Madison, USA). DPPH radical scavenging activity (% inhibition, % I) of SEOs was calculated using the following equation (Gourine et al., 2010):

$$\%I = (1 - A_S/A_B) \times 100\%,$$

where $A_S$ and $A_B$ were A values of samples and blank, respectively. Gallic acid (GA monohydrate, Carl Roth, Germany) solutions (0–100 μg/ml methanol) was used as standards ($R^2$ 0.9025, y = 23.75 X − 33.75). From preliminary experiments, up to 10 μg/ml menthone (90%, Acros organics, India) had no DPPH radical scavenging activity, and thus was included as a negative reference. Concentrations of SEOs providing 50% inhibition ($IC_{50}$) of DPPH were calculated from a plot of sample concentrations and % inhibition ($R^2$ 0.871, Y = 11.25X − 14.375). The test was conducted in triplicates.

### 2.7. Statistical analysis

Results are means of triplicate experiments. One-way analysis of variance (ANOVA) was used and mean difference was considered significant at P ≤ 0.05. Means were separated by the Duncan's test (SPSS 13, SPSS Inc., Chicago, Il., USA). Student's *t*-test was applied to determine significance differences between samples and reference antibiotic.

## 3. Results and discussion

EOs are volatile secondary metabolites of aromatic plants, having a strong odor and are water insoluble. They are usually obtained by hydro-distillation first developed in middle ages by the Arabs (Bakkali et al., 2008; Dima and Dima, 2015). EOs exhibited antibacterial, antifungal, antiparasitic, and antiviral activities. EOs extracted from plants have been used as bio-functional components for centuries as part of natural traditional medicine (Daglia, 2012).

### 3.1. Physicochemical characteristics of SEOs

Table 1 lists some physicochemical properties of SEOs. Fresh spathe distillation process produced 0.042% (dwb) SEOs. Similar extraction % yields were reported (Demirci et al., 2013; Mohamadi et al., 2014). Variations in % yields could be attributed, among others, to plant species, geographical locations, growth conditions and distillation parameters. SEOs had a bright yellow color with pleasant aroma. Density and refractive index of oils were 0.987 and 1.5905, respectively (Table 1). Density, refractive index and other physicochemical properties of SEOs have not previously been reported. Physical properties such as color, density and refractive indices are among the physical properties to assess purity, consumer safety and fair trade of EOs (Ud-Daulaa et al., 2016; Do et al., 2015). Falsifications of EOs negatively affect their safety and quality, especially when added to food, pharmaceuticals and cosmetics. EOs could be adulterated by using

**Table 1**
Yield and some physicochemical characteristics of essential oils extracted from date palm spathe.

| Property | Value |
|---|---|
| Yield (%, dwb)[a] | 0.042 |
| Color | Bright yellow |
| Density at 23 °C | 0.987 |
| Refractive index (23 °C) | 1.5905 |

[a] Dry weight basis.

**Table 2**
GC–MS analysis of spathe essential oils (SEOs).

| Peak no. | Retention time (min) | Ret. index | Component | Concentration (% area) |
|---|---|---|---|---|
| 1 | 5.225 | 1083 | Octanoic acid, methyl ester | 0.019 |
| 2 | 5.458 | 1120 | 2,6-Nonadienal | 0.025 |
| 3 | 5.75 | 1173 | Octanoic acid | 0.223 |
| 4 | 5.975 | 2263 | 3-Fluorobenzoic acid | 0.124 |
| 5 | 6.133 | 1143 | β-Terpineol | 0.372 |
| 6 | 6.342 | 1172 | 2,3-Dimethoxytoluene | 4.138 |
| 7 | 6.542 | 1172 | 2,6-Dimethoxytoluene | 3.813 |
| 8 | 7.1 | 1172 | 3,4-Dimethoxytoluene | 38.278 |
| 9 | 7.2 | 1172 | 2,6-Dimethoxytoluene | 5.224 |
| 10 | 7.425 | 1190 | Anisole | 1.329 |
| 11 | 7.567 | 1248 | Methylsyringol | 0.802 |
| 12 | 7.789 | 1361 | 1,2,3-Trimethoxy-5-meyhyl benzene | 3.549 |
| 13 | 8.025 | 1311 | 2-Undecenal | 0.117 |
| 14 | 8.208 | 1248 | 1.2,4-Trimethyl benzene | 0.292 |
| 15 | 8.292 | 1311 | 2-Undecenal | 0.090 |
| 16 | 8.483 | 1372 | n-Decanoic acid | 0.267 |
| 17 | 8.642 | 1361 | 3,4,5-Trimethoxy toluene | 0.150 |
| 18 | 8.875 | 1402 | Dodecanal | 0.123 |
| 19 | 9.033 | 1361 | 1,2,3-Trimethyl benzene | 1.279 |
| 20 | 9.117 | 1429 | α-Ionone | 0.553 |
| 21 | 9.217 | 1494 | Carophyllene | 0.700 |
| 22 | 9.375 | 1420 | 5,9-Undecadien-2-one | 12.506 |
| 23 | 9.608 | 1579 | α-Carophyllene | 0.231 |
| 24 | 9.792 | 1457 | β-Ionone | 0.742 |
| 25 | 10.008 | 1576 | Dicyclohexyl methanone | 0.074 |
| 26 | 10.103 | 1426 | 2(4H)-benzofuranone | 0.106 |
| 27 | 10.808 | 1570 | Dodecanoic acid | 0.357 |
| 28 | 10.908 | 1280 | Cyclopropanemethanol | 0.125 |
| 29 | 11.025 | 1507 | Carophyllene oxide | 0.853 |
| 30 | 11.183 | 1579 | α-Carophyllene | 0.015 |
| 31 | 11.25 | 1603 | Benzophenone | 0.259 |
| 32 | 14.467 | 1902 | Farnesyl acetone | 1.307 |
| 33 | 14.608 | 1968 | Palmitic acid | 0.814 |
| 34 | 15.117 | 1968 | n-Hexadecanoic acid | 4.583 |
| 36 | 16.717 | 2175 | 9-Octadecenoic acid | 5.374 |
| 37 | 16.906 | 2167 | Octadecanoic acid | 1.548 |
| 38 | 17.283 | 2183 | 9,12-Octadecadienoic acid | 0.790 |
| 39 | 19.508 | 2498 | Hexadecanoic acid | 2.398 |
| 40 | 20.942 | 2689 | 9-Octadecenoic acid (*Z*)-, 2,3-dihydroxypropyl ester | 3.134 |
| 41 | 21.175 | 2681 | Octadecanoic acid, 2,3-dihydroxypropyl ester | 1.507 |
| Total identified | | | | 98.189 |

inauthentic herbs or mixing with vegetable oils (Dima and Dima, 2015).

### 3.2. GC–MS analysis of SEOs

Many EOs have been chemically classified by GC–MS and monographed by the European pharmacopoeia, ISO and WHO to ensure their quality (Bakkali et al., 2008). Composition of EOs depend on plant species extracted, geographic regions, time of harvest, stage of development and processing techniques (Dima and Dima, 2015; Ribeiro-Santos et al., 2017). Qualitative and semi-quantitative (% area) GC–MS of EOs have been used to shed light on oil constituents (Jakab et al., 2018). GC–MS data of SEOs are presented in Table 2. Additionally, the total ion chromatogram (TIC) of SEOs is seen in Fig. 1. Forty one constituents (> 0.01%) of SEOs were identified, representing 98.2% of total oil (Table 2). Major compounds of SEOs were 3,4-dimethoxytoluene (38.28%) and its isomers. Other major constituents were 5,9-Undecadien-2-one (12.51%), 9-Octadecenoic acid (5.37%) and n-hexadecanoic acid (4.58%) (Table 2). 3,4-Dimethoxytoluene, as a dominant compound, corroborated findings of previous reports on SEOs (Demirci et al., 2013; Mohamadi et al., 2014). However, only sixteen constituents were identified, of which 3,4-dimethoxytoluene represented 73.5% of 99.03% total EOs (Demirci et al., 2013). Interestingly, 95% of total SEOs were 3,4-dimethoxytoluene (Al Yahya, 1986). Discrepancies in concentration of 3,4-dimethoxytoluene among studies could be related to geographic sources of samples, environmental conditions, sample freshness, distillation parameters and analytical procedures. EOs are a mixture of bioactive components (*e.g.*, terpenes and phenyl terpenes and phenylpropenes). The activity of components, structure and hydrophobicity of functional groups were responsible for biological function of EOs (Gyawali and Ibrahim, 2014; Harich et al., 2018). Biological activity of EOs depended on effects of all components and their interactions. In fact, few constituents could make up to 85% of EOs and thereby contributed to the primary property of the mixture (Miguel, 2010; Zhai et al., 2018). In this regard, 3,4-dimethoxytoluene, its isomers and 5,9-Undecadien-2-one (geranylacetone) accounted for > 60% of SEOs (Table 2). Research works have been focusing on developing innovative techniques to extract and stabilize EOs (Dima and Dima, 2015). EOs formulations have been used in food processing (antioxidants and antimicrobials) and packaging and are GRAS in view of their safety profile. Use of EOs has been regulated by the European Union Cosmetics Regulation, European Food Safety Authority (EFSA) and other concerned establishments (Do et al., 2015). Depending on the food type, the maximum quantity of EOs permitted ranged between 6.62 and 62.06 mg/kg (Dima and Dima, 2015; Zantar et al., 2014). EOs have also been applied in pharmaceuticals and cosmetics industries.

#### 3.2.1. Inhibition activity of SEOs

The present study is the first report on the inhibitory activity (*in vitro*) of SEOs against *L. monocytogenes* ATCC 7644 and other reference (ATCC strains) pathogens (Table 3). The agar well assay (*in vitro*) is a screening technique used for qualitative assessment of the inhibitory activity of test materials (*e.g.* EOs) against indicator microorganisms.

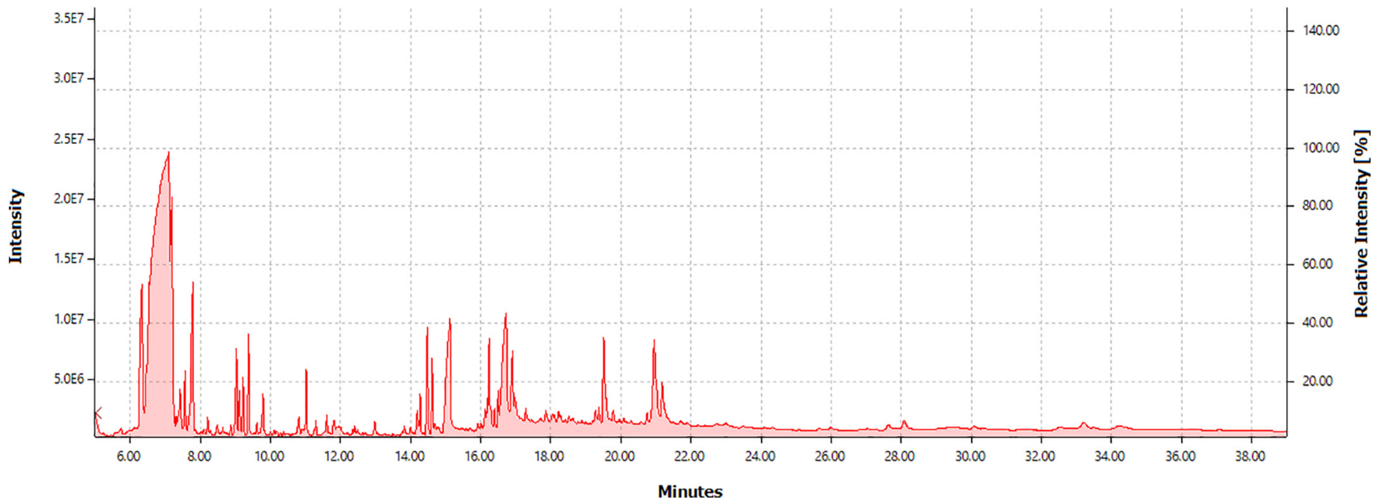


**Fig. 1.** Total ion chromatogram (TIC) of essential oils from spathe (SEOs).

**Table 3**
*In vitro* antibacterial activity (inhibition zones) of spathe essential oils (SEOs) against some pathogens.

| Bacteria | Inhibition zone (mm) | | |
|---|---|---|---|
| | SEOs | Distillates/hydrosol | Reference[a,*] |
| *L. monocytogenes* ATCC 7644 | 13 | –[c] | 22 |
| *Staphylococcus aureus* ATCC 29213 | 11 | – | 25 |
| *Escherichia coli* ATCC 25922 | 12 | – | 20 |
| *Salmonella enterica* ATCC 13076 | (12)[b] | – | 28 |

[a] Pristinamycin IA (20 μg/ml).
[b] Hazy zone (not clear).
[c] No inhibition zone.
[*] $P < 0.05$ (Student's *t*-test).

Pristinamycin IA gave wider zones of inhibition, compared to SEOs (Table 3). Zone diameters afforded by SEOs were 11–13 mm, depending on challenged bacteria. SEOs gave hazy zones (not clear) against *Salmonella enterica* ATCC 13076, which may indicate a bacteriostatic effect (Table 3). SEOs was active against both gram positive (*L. monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 29213) and gram negative (*Salmonella enterica* ATCC 13076 and *Escherichia coli* ATCC 25922) bacteria. It is of note that *L. monocytogenes* ATCC 7644 is a clinical isolate causing listeriosis which is a severe food-borne illness resulting in meningitis, sepsis and gastrointestinal diseases. As a matter of fact, listeria infections may result in abortion or septic illness of newborns. Besides, it has a high mortality rate (above 30%) and thus it is considered as a serious public health concern (Longhi et al., 2003; Albonetti et al., 2017). The other gram-positive bacteria sensitive to SEOs was *Staphylococcus aureus* ATCC 29213 (Table 3). That staphylococcal strain was reported to produce A, C and E exotoxins (Azizkhani et al., 2013). It should be noted that SEOs was active against standard strains (*E. coli* ATCC 25922, *Salmonella enterica* ATCC 13076 and *Staphylococcus aureus* ATCC 29213). Those pathogens have been adopted by the Clinical and Laboratory Standards Institute (CLSI) for the antimicrobial susceptibility testing (Ahumada-Santosa et al., 2016). *In vitro* antimicrobial activity of different EOs against some spoilage and pathogenic microorganisms was published by Burt (2004).

Hydrosols are secondary products of hydro-distillation for aromatic herbs and spices. Hydro-distillates and Hydrosols of fresh spathe lacked any activity against four pathogens tested (Table 3). That may indicate that only n-hexane fractions of fresh spathe contained higher concentrations of biologically active substances. Hydrosols of some thyme and oregano species had antibacterial activity (disk assay) against *E. coli* O157:H7, *Staphylococcus aureus* and other bacteria (D'Amato et al., 2018). It is worthwhile to mention that hydrosols (aromatic water) of selected plants have been used for medical purposes and for enhancing flavor of drinks and other foods (Demirci, et al., 2013; Hamedi et al., 2013; D'Amato et al., 2018).

### 3.2.2. Inhibitory activity of SEOs in food model

EOs had broad spectrum *in vitro* activity against both gram negative and gram-positive food-borne pathogens. However, few information exists on the practical use of such antimicrobial extracts in food systems, such as meat. Poultry meat (*e.g.* chicken) is a popular food owing to its low cost, reduced fat content and high nutritional values. However, the poultry industry has been concerned about safety of such a perishable food (Chouliara et al., 2007; Aziz and Karboune, 2018). Poultry meat could be naturally contaminated with *Staphylococcus aureus*, *Salmonella enterica* and other pathogens (Stojanovic-Radic et al., 2018). Therefore, that would result in food-borne illnesses and economical losses. Hazard analysis and critical control point (HACCP) programs have been adopted (compulsory) to insure poultry meat safety (Stojanovic-Radic et al., 2018). FDA of the USA approved use of chemicals, such as lactic acid for decontaminant of poultry meat (Zhu et al., 2016). Information availability and accessibility on health and quality have resulted in persistent consumer demands for more safe and better-quality food products. Therefore, the food industry, research communities and regulatory authorities have been motivated to search for natural alternatives for chemical food additives. As a result, the present study aimed to evaluate the potential antibacterial activity of a natural extract (SEOs) in a food product (chicken meat). The inhibitory activity of SEOs was dose dependent (Table 4). SEOs (1%) had a

**Table 4**
Inhibition ($\log_{10}$ cfu/g) by spathe essential oils (SEOs) of two bacterial pathogens in chicken meat stored at 20 °C for 18 h.

| SEOs (%) | $\log_{10}$ cfu/g | |
|---|---|---|
| | *Staphylococcus aureus* ATCC 29213 | *Escherichia coli* ATCC 25922 |
| 0 (control)[α] | 7.49[a] | 8.81[a] |
| 0.125 | 3.43[b] | 5.46[b] |
| 0.5 | 3.20[b] | 3.69[c] |
| 1 | –[≠] | – |
| α-Terpineol (1%) | – | – |

a–c means in a column without common superscripts are significantly ($P < 0.05$) different.
[α] Zero-time count (3 $\log_{10}$ cfu/g).
[≠] –, below detection limit (< 1 $\log_{10}$ cfu/g).

bactericidal activity (< 1 $\log_{10}$ cfu/g) on *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 in meat samples subjected to abusive storage conditions (20 °C, 18 h). In fact, storage at or below 4 °C is a critical control point (CCP) of HACCP in poultry meat industry. Temperature disturbances and chilling defects result in compromising of meat safety and product losses. The application of the lowest SEOs (0.125%) used in this study was sufficient to limit the increase of *S. aureus* to only 0.4 $\log_{10}$ cfu/g during 18 h storage at 20 °C, compared to the initial inoculated level (Table 4). *Staphylococcus aureus* ATCC 29213 produced exotoxins causing gastroenteritis (Azizkhani et al., 2013). On the other hand, *Escherichia coli* ATCC 25922 was less sensitive to 0.125% SEOs than the above gram-positive bacterial pathogen (Table 4). *Escherichia coli* ATCC 25922 challenged with 0.5% SEOs in chicken meat was only 0.7 $\log_{10}$ cfu/g higher in numbers than zero time count of control (Table 4). Meanwhile, *Escherichia coli* ATCC 25922 significantly ($P < 0.05$) increased in cell count to approximately 9 $\log_{10}$ cfu/g in control (Table 4). The resistance of gram-negative bacteria to EOs and to other antimicrobial substances was related to lipopolysaccharide (LPS) membranes surrounding cell walls of gram-negative ones (Dussault et al., 2014; Aziz and Karboune, 2018; Stojanovic-Radic et al., 2018). It is worthwhile to mention that *E. coli* ATCC 25922 has been used as a surrogate for pathogenic *E. coli* including *E. coli* O157:H7 (causes hemorrhagic colitis) in industrial testing protocol, as recommended by the AOAC and other regulatory agencies (Cook et al., 2017). In fact, non-pathogenic *E. coli* strains could be used as surrogates for thermal processing verification and validation of non-typhoidal *Salmonella* in meat (Redemann et al., 2018).

The antibacterial activity of SEOs could be attributed to biologically active constituents detected by GC–MS analysis (Table 2). 3,4 Dimethoxytoluene, its isomers and 5,9-Undecadien-2-one (geranyl acetone) were dominant constituents in SEOs (Table 2). Those major compounds exhibited antibacterial activity to *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 2392, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 15442 and other organisms (Francis and Jose, 2016; Haque et al., 2016; Jablonsky et al., 2017). EOs components caused structural damages in microorganisms by diffusion through the cellular membrane and this ability is due to the hydrophobic nature of volatile oils (Lambert et al., 2001).

To our knowledge, the present investigation is the first attempt in evaluating the inhibitory activity of SEOs against intentionally contaminated food (chicken meat) with serious human pathogens. Chicken meat samples treated with 1% oregano EOs and refrigerated in modified atmospheric packaging (MAP) had longer shelf life (Chouliara et al., 2007; Aziz and Karboune, 2018). Met cubes contained 500 ppm commercial EOs provided up to 20% inhibition of mixed listeriae. 500 ppm EOs was the maximum acceptable sensory limit for product (Dussault et al., 2014). More than ten folds (> 5000 ppm) was the minimum inhibitory concentration for twenty EOs in broth cultures of *L. monocytogenes*, *E. coli* and *Staphylococcus aureus* (Dussault et al., 2014). In fact, meat cube formulations (Dussault et al., 2014) contained 200 ppm nitrite ($NO_2$), 1.5% NaCl and phosphates. As a result, $NO_2$ (potential carcinogen) and salts may have potentiated the antilisterial activity of the lower concentration (500 ppm EOs) in meat cubes. It should be noted that poultry meat deteriorated after four to ten days under chilling conditions (Samapoundo et al., 2018). Meat preservation using reduced EOs concentrations may be achieved through their application as part of a hurdle system. For instance, 0.1% EOs could be combined with other preservation technologies such as low temperature and MAP (Dussault et al., 2014; Jayasena and Jo, 2014). Limited quantities of applied EOs have been considered for natural complex which are safe under intended conditions of use (Dima and Dima, 2015). Chicken meat treated with 0.1% oregano EOs prior to microwave cooking had good scores for odor and taste (Chouliara et al., 2007). Dipping in basil EOs resulted in acceptable sensory scores (> 5) of oven cooked chicken meat (Stojanovic-Radic et al., 2018). Substantial differences in the inhibition of *Salmonella* by 0.5% EOs of basil, rosemary or combinations were evident between two chicken meat models (autoclaved or flame sterilized plus UV treatment). EOs tested performed better against the pathogen in autoclaved meat samples stored at 18 °C for three days. It was clearly concluded that intrinsic factors of autoclaved meat, including lower water availability potentiated anti-salmonella in samples soaked in 0.5% EOs (Stojanovic-Radic et al., 2018). Although 0.5% basil, rosemary or their combination had significant effects ($P < 0.05$) on *Salmonella enteritidis*, pathogen counts were ~1 to 5 $\log_{10}$ cfu/g higher than the initial counts (4 $\log_{10}$ cfu/g) in both chicken models and under either 4 or 18 °C for up to 72 h (Stojanovic-Radic et al., 2018). In fact, 0.5% SEOs maintained counts of the gram-negative or gram-positive bacteria 0.7 and 0.2 $\log_{10}$/g, respectively, higher than the zero time counts after 18 h at 20 °C of meat storage (Table 4). Concerning poultry feeding, the growing concern over the transmission of resistant bacteria *via* the food chain has led to a ban on antibiotic growth promoters in feed within the European Union. Thus, EOs could be better alternatives in feeding strategies of poultry (Brenes and Roura, 2010).

### *3.3. DPPH radical scavenging activity (RSA) of SEOs*

Although many studies (*e.g.*, Cohena et al., 2018; Aziz and Karboune, 2018) reported on the antioxidant activity of EOs, this is the first investigation on the antioxidant capability of SEOs. The antioxidant activity of SEOs was evaluated and compared to that of gallic acid (Table 5). RSA of SEOs increased in a concentration-dependent pattern (Table 5). In fact, above 0.63 µg/ml, % RSA increased but was not significantly ($P > 0.05$) different (Table 5). In addition to concentrations, steric factors influenced RSA of antioxidants (Gourine et al., 2010). SEOs concentration providing 50% inhibition ($IC_{50}$) of DPPH was 0.61 µg/ml (Table 5), as calculated from linear regression equation of SEOs against % inhibition of DPPH. Lower $IC_{50}$ indicated higher RSA. In Table 2, SEOs contained major constituents (3,4-dimethoxytolun, its isomers, *etc.*) having antioxidant abilities. In this regard, 3,4-dimethoxytolune and caryophyllene oxide possessed antioxidant activities (Haque et al., 2016; Jablonsky et al., 2017). The long history of safe use of EOs and limited quantity of applied EOs have been considered for natural flavor complex which are safe under intended conditions of use (Dima and Dima, 2015). Lipid oxidation in food is a detrimental chemical reaction affecting food quality. Reactive oxygen species (ROS) cause damage to biological molecules. Oxidation stress could result in cardiovascular diseases, cancer, aging and other adverse disorders. Antioxidants play a major role in delaying or preventing formation of ROS. EOs exhibited DPPH radical scavenging activity could exert lipid stabilization in different meat types (Stojanovic-Radic et al., 2018). As a result, consumers and research groups seeking more safer food has motivated the search for natural antioxidants for food products. It is clearly stated that natural antioxidants could not only extend the shelf-life of food, but also have health benefits (Aziz and Karboune, 2018). Date palm trees are natural sources for active constituents (*i.e.*, SEOs) which could preserve quality and maintain safety of some food products. SEOs could be an added-value product which

**Table 5**
DPPH radical scavenging activity (% inhibition) and $IC_{50}$ of SEOs.

| SEOs/reference (µg/ml) | % inhibition | $IC_{50}$ (µg/ml) |
|---|---|---|
| 0.16 | 45.48122[c] | 0.61 |
| 0.31 | 57.92254[b] | |
| 0.63 | 71.3615[a] | |
| 1.25 | 73.18075[a] | |
| 2.5 | 77.11268[a] | |
| Gallic acid | | 2.95 |
| Menthone (0.625–10) | –[≠] | |

a–c, means without common superscripts are significantly ($P < 0.05$) different.
≠ No inhibition.

may be employed in the food industry and pharmaceuticals.


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