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Antimicrobial and antioxidant activities of red onion, garlic and leek in sausage

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This study was designed to evaluate antimicrobial and antioxidant effect of ethanolic and aqueous extracts and essential oils of red onion, garlic and leek against *Escherichia coli* O₁₅₇:H₇, *Staphylococcus aureus*, A.F., 4, *Salmonella typhimurium*, A.F., 3, *Aspergillus niger*, H.U.B., 1, *Aspergillus ochreus*, H.U.B., 12 and *Fusarium oxysporum*, H.U.B., 3 in sausage. The susceptibility of these isolates toward the extracts of these plants was compared with some antibiotics (oxytetracycline, tetracycline, ampicillin and amoxicillin) used as positive control. The phenolic contents and stable free radical 2,2-diphenyl-1-picrylhydrazyl was determined. Results show that the concentration, 60 mg/ml of ethanolic extracts and essential oils represented the optimum concentration against all microorganisms. The essential oils exhibited higher effect than ethanolic and aqueous extracts against all tested microorganisms, especially at 60 mg/ml concentration of leek essential oils, whereas red onion, garlic and leek essential oils showed a stronger antimicrobial activity for decreasing count of *E. coli* O₁₅₇:H₇, *S. aureus*, A.F., 4 and *Salmonella typhimurium*, A.F., 3 in sausage. The phenolic contents and antioxidant activity were higher in essential oils of garlic, leek and red onion whereas the lower contents and activity were shown in aqueous extracts. The essential oils of red onion had the highest phenolic contents and antioxidant activity in contrast to garlic essential oils.

Key words: Microorganisms, antioxidant, essential oils, ethanolic, aqueous extracts, onion, garlic, leek.

INTRODUCTION

The presence of spoilage microorganisms in food can accelerate the lipid oxidation and other oxidation processes, or can produce changes in the organoleptic properties of the foods, specially the fungi that can give some characteristic color to the food due to its growth (Saggiolato et al., 2012). Most of the foods borne bacterial pathogens are sensitive to extracts from plants such as garlic, mustard, onion and oregano. Gram-

positive bacteria are more sensitive to antimicrobial compounds in spices than Gram-negative bacteria (Lawson, 1996). Essential oils extracts have been considered as natural preservatives or food additives, and can be used as additional methods of controlling pathogens (Naidu, 2000). Besides the antibacterial, antifungal and anti-inflammatory activities, many essential oils (Eos) also have been confirmed to possess antioxidant activity

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(Elaiissi et al., 2011; Prakash et al., 2012; Viuda-Martos, et al., 2010).

Onion (*Allium cepa* L.), garlic (*Allium sativum* L.), leek (*Allium porrum*) and other edible *Allium* are among the oldest cultivated plants, and all belong to a family of Alliaceae. They are a rich source of several phytonutrients, and recognized to have significant and wide biological activities (Benkeblia and Lanzotti, 2007). Onion is commonly used as a spice in Turkey especially in ground beef, doner kebab, meat ball and raw meat balls-cig kofte; it may also be used to reduce pathogenic microorganisms contamination during unhygienic productions (Degirmencioglu and Irkin, 2009). Onion aqueous extracts are effective against many yeast species and several G (+) bacteria but ineffective against G (-) bacteria (Benkeblia, 2004; Ghahfarokhi- Shams et al., 2006). A strong antimicrobial effect of fresh onion homogenates was due to both methyl cysteine sulfoxide and S-n-propyl cysteine sulfoxide from which the corresponding thio-sulfonates are formed enzymatically (Kyung and Lee, 2001). Onion is an important food because it supplies various activated phytochemicals such as phenolic acid, flavonoids, copanenes, thiosulfinate, organosulfur compounds (OSCs), and anthocyanin (Slimestad et al., 2007). Among the species of onions, the red onion is abundant in polyphenols, flavonoids, flavonol and tannin (Gorinstein et al., 2010). The essential oil from *A. cepa* may be a new potential source of natural antimicrobial and antioxidant agents applied in food systems (Ye et al., 2013).

Garlic is one of the most commonly used ingredients as a flavor enhancer for sausage (Harris et al., 2001). Garlic is effective against bacteria, protozoa, fungi and some viruses (Jaber and Al-Mossawi, 2007). Thiosulfonates play a major role in the antibiotic activity of garlic (Durairaj et al., 2009). Addition of fresh garlic and garlic powder produced significant antioxidant and antimicrobial effects and extended the shelf-life during refrigerated storage (Dewi et al., 2010).

Leek extracts showed positive antioxidant activities and positive antimicrobial activities (Kyoung-Hee et al., 2012). Aqueous extract of *A. porrum* had antimicrobial activity against six bacterial species: three Gram-positive bacteria (*Bacillus subtilis*, *Streptococcus pneumoniae* and *Staphylococcus aureus*) and three Gram-negative bacteria (*Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa*). The biologically active components include amino acid with sulphate, glycolipids, saponin and phenol present in the aqueous extract (Naem-Rana and Hadi-Noora, 2012).

Aspergillus species are the most common fungal species which are able to produce mycotoxins in food and feed stuffs and these mycotoxins are known to be potent hepatocarcinogens in animal and humans (Soliman and Badaea, 2002). The presence and growth of fungi may cause spoilage and result in reduction in quality and quantity of foods (Rasooli and Abyaneh, 2004).

The purpose of this study was to investigate the anti-

bacterial and antifungal activities of onion, garlic and leek against some pathogenic bacteria and fungi *in vitro* and in beef sausage. Also, the total phenolic contents and antioxidant properties of studied plant materials were assessed.

MATERIALS AND METHODS

Plant materials

Fresh garlic (*A. sativum* variety), leek (*A. porrum* variety) and red onion (*Allium cepa* variety) were purchased from the local market, Cairo, Egypt. They were peeled, sliced, and dried in an air dry oven at 40°C, then ground to a fine powder using an electrical mill, and kept in polyethylene bags in a refrigerator at 4°C for further analysis.

Microorganisms

Three pathogenic bacterial strains (*E. coli* O₁₅₇: H₇, *S. aureus*, A.F., 4 and *S. typhimurium*, A.F., 3) and three pathogenic fungal strains (*A. niger*, H.U.B., 1, *Aspergillus ochreus*, H.U.B., 2 and *Fusarium oxysporum*, H.U.B., 3) were used throughout this study. Bacterial strains were obtained from Dr Abdel-Salam. A.F., Regional Center for Food and Feed, ARC, Giza Egypt. Fungal strains were obtained from Biotechnology Department, Helioypolis University, Cairo, Egypt. The bacteria were selected because they are frequently reported in food spoilage, while the selected fungi are commonly encountered in onions and responsible for bulb diseases. The isolates were stored at 4°C. The symbols and numbers of the end of microbial synthetic name are code numbers for microbial registration of each strain.

Essential oils extraction

The dried samples were subjected to steam distillation using method of Aqel and Shaheen (1996). 10 g of powdered sample were added to 200 ml distilled water and extraction was carried out by steam distillation. The process continued until about 200 ml of distillate was collected. The distillate was extracted 3 times with chloroform. After removing moisture by using anhydrous sodium sulphate, the extract was evaporated on a water bath (40°C). Stock solutions of crude essential oils were prepared by diluting the dried essential oils with 10% dimethyl sulphoxide (DMSO) solution.

Preparation of aqueous and ethanolic extracts

Ten grams of each dry powder samples were extracted with 100 ml of 80% ethanol or distilled water in a screw-capped flask and shaken at room temperature for 24 h. The extracts were filtered through Whatman paper (No. 1) and the solvent was removed using rotary vacuum evaporator at 40°C, then the concentrated extracts were restored in a freezer at -20°C until analyzed. Stock solutions of crude extracts were prepared by diluting the dried extracts with dimethyl sulphoxide (DMSO) solution.

Preparation of inoculum

Bacterial inoculum was prepared by growing in Brain-Heart Infusion broth (Merck, Darmstadt, Germany) for 24 h at 37°C. All bacteria tested were enumerated by using the serial dilution method on specific selective agar for each strain.

Fungi was cultured on potato dextrose agar (PDA) medium for 7-10 days at 30°C, and then fungi suspension for each strain was prepared.

Preparation of sausage

Beef meat, animal fat, sheep casing and salt were purchased from local market. Sausages were manufactured according to the following traditional formula, 55% lean beef meat and a maximum of about 25% animal fat, 3% salt and 21% ice water. This mixture additives was added, and filling in casings using filler (Moulinex, France) according to the method of Nowak et al. (2007). Sausages were examined for presence of *E. coli* O₁₅₇: H₇, *Saureus* and *Salmonella typhimurium*.

Antibacterial and antifungal activity

The plant extract (ethanolic, essential oils and aqueous) were prepared from onion, garlic and leek at different concentrations (20, 40 and 60 mg/ml) and antibiotics (oxytetracycline, 30 µg; tetracycline, 30 µg; ampicillin 10 µg and amoxicillin, 25µg) then tested for antibacterial and antifungal activity. Antibiotics were used for the comparison with the plant extractions.

Agar disc diffusion method

The agar disc diffusion method is the most widespread technique of antibacterial and antifungal activity assessment. This method is normally used as a preliminary check and to select the best efficient against pathogenic bacteria and fungi (Nedorostova et al., 2009). Nutrient Agar was used for *E. coli* O₁₅₇: H₇, *S. aureus* and *S. typhimurium* after inoculated with bacterial inoculums. Potato dextrose agar (PDA) was used for *A. niger*, *A. ochreus* and *F. oxysporum* after inoculation with fungal inoculum.

The microorganisms (bacteria or fungi) and growth media were mixed thoroughly to ensure uniform distribution of the microorganisms. The sterile filter discs (Whatman No 1, Maidstone, England, 6 mm diameter) were impregnated with 30 µl of each dilution to each filter paper disc and placed on the agar surface using forceps dipped in ethanol and flamed. All Petri dishes were sealed with sterile laboratory parafilm and left for 30 min at room temperature to allow the diffusion of extractions. Antibiotics discs were placed on the agar surface with the same media mentioned before for bacteria and fungi using forceps dipped in ethanol and flamed also. Plates for antimicrobial activity test were incubated at 37°C for 24-48 h. Plates for antifungal activity test were incubated at 30°C for 7-10 days. After the incubation period, the mean diameter of inhibition halo where test microorganism did not grow (clearly visible inhibition zone) was measured in millimeter, for each disc and evaluated for susceptibility or resistance (Konman et al., 1997).

Application effect of essential oils on growth of *Escherichia coli* O₁₅₇: H₇, *S. aureus* and *S. typhimurium* in beef sausage

Sausage samples were divided into nine groups (the group composed of spices with 25 g each) three groups dipped in *E. coli* O₁₅₇: H₇ culture (about 10⁸ cfu/ml) for 5 min, three groups dipped in *S. aureus* culture (about 10⁸ cfu/ml) for 5 min and another three groups dipped in *S. typhimurium* culture (about 10⁸ cfu/ml) for 5 min and then left all inoculated groups with *E. coli* O₁₅₇:H₇ or *S. aureus* or *S. typhimurium* for 10 min under aseptic condition before initial cfu/g was determined, then dipped in different essential oils of onion, garlic and leek at 40 mg/ml concentration for 15 min and the

mean cfu/g was determined for *E. coli* O₁₅₇: H₇, *S. aureus* and *S. typhimurium*.

Determination of total phenolic contents

The total phenolics in the extracts were estimated by spectrophotometric assay (Barreira et al., 2008). One milliliter of sample (concentration 1 mg/mL) was mixed with 1 mL of Folin and Ciocalteu's phenol reagent. After 3 min, 1 mL of sodium carbonate solution (7.5%) was added to the mixture and made up to 10 mL with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm in a spectrophotometer against a blank sample. Gallic acid was used for constructing the standard curve (20-100 µg/mL) and the results were expressed as µg of gallic acid equivalents (GAE)/mg of extract and the values are presented as means of triplicate analyses.

Determination of the scavenging activity by using DPPH

The radical scavenging activity of antioxidants of plant essential oils, ethanolic extracts and water extracts against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was measured using the method of Barros et al. (2007). 300 µl of samples were taken in different test tubes. 2.7 ml of 6 × 10⁵ mol/l DPPH solution made up with DPPH (4.8 mg) in methanol (200 ml) was added to these tubes. The mixture was shaken and left to stand at room temperature in the dark for 90 min. Absorbance of the resulting solution was measured at 517 nm by a UV visible spectrophotometer. The readings were compared with controls, which contained 300 µl of methanol instead of the extract. Methanol was used as blank. Radical scavenging activity (RSA) was expressed as the inhibition percentage and was calculated using the following equation:

$$\text{RSA (\%)} = [(A_{\text{DPPH}} - A_{\text{S}}) / A_{\text{DPPH}} \times 100]$$

Where, A_S is the absorbance of the solution when the sample extract is added and A_{DPPH} is the absorbance of the DPPH solution.

Statistical analysis

Duncan's multiple range test was used to test significance of means of 5 replicates of samples according to IBM ® SPSS ® Statistics software (IBM, 2011).

RESULTS AND DISCUSSION

Red onion, garlic and leek extracts exhibited different inhibition levels against *E. coli* O₁₅₇: H₇, *S. aureus*, A.F., 4, *S. typhimurium*, A.F., 3, *A. niger*, H.U.B., No.1, *A. ochreus*, H.U.B., 2 and *F. oxysporum*, H.U.B., 3 as shown in Tables 1, 2 and 3. In the dose response study, the inhibition zone increased with increasing concentration of extracts.

Inhibition zone (mm) of red onion extracts (mg/ml) on different microorganisms

The data recorded in Table 1 obviously showed that *S. typhimurium*, A.F., 3 was more sensitive to ethanolic and

Table 1. Inhibition zone (mm) of red onion extracts (mg/ml) on different microorganisms.

| Microorganism | Plant extract | | | | | | | | | | | |
|---|----------------|-----------------|------------------|------------------|----------------|------------------|------------------|-----------------|----------------|-----------------|-----------------|------------------|
| | Control | Ethanolic | | | Control | Essential oil | | | Control | Aqueous | | |
| | | 20 | 40 | 60 | | 20 | 40 | 60 | | 20 | 40 | 60 |
| <i>E. coli</i> O ₁₅₇ :H ₇ | 5 ^e | 8 ^d | 10 ^c | 13 ^b | 7 ^d | 11 ^c | 13 ^b | 15 ^a | 0 ^f | 0 ^f | 8 ^d | 10 ^c |
| <i>S. aureus</i> , A.F., 4 | 6 ^e | 9 ^d | 11 ^{cd} | 14 ^b | 9 ^d | 12 ^c | 14 ^b | 16 ^a | 0 ^f | 0 ^f | 9 ^d | 11 ^{cd} |
| <i>S. typhimurium</i> , A.F., 3 | 5 ^f | 10 ^d | 12 ^c | 15 ^b | 7 ^e | 13 ^c | 16 ^b | 18 ^a | 0 ^g | 0 ^g | 9 ^d | 12 ^c |
| <i>A. niger</i> , H.U.B., 1 | 5 ^e | 7 ^d | 9 ^{bc} | 10 ^{ab} | 5 ^e | 8 ^{cd} | 10 ^{ab} | 11 ^a | 0 ^f | 0 ^f | 0 ^f | 0 ^f |
| <i>A. ochreus</i> , H.U.B., 2 | 5 ^e | 8 ^d | 10 ^{bc} | 11 ^b | 5 ^e | 9 ^{cd} | 11 ^b | 13 ^a | 0 ^f | 0 ^f | 0 ^f | 0 ^f |
| <i>F. oxysporum</i> , H.U.B., 3 | 5 ^e | 9 ^{cd} | 10 ^{bc} | 12 ^{ab} | 6 ^e | 10 ^{bc} | 11 ^{ab} | 13 ^a | 0 ^f | 7 ^{de} | 9 ^{cd} | 10 ^{bc} |

Values in the same row followed by the same letter (s) do not significantly differ from each other according to Duncan's at 5% level.

Table 2. Inhibition zone (mm) of garlic extracts (mg/ml) on different microorganisms.

| Microorganism | Plant extract | | | | | | | | | | | |
|---|----------------|------------------|------------------|------------------|-----------------|------------------|-----------------|-----------------|----------------|------------------|------------------|------------------|
| | Control | Ethanolic | | | Control | Essential oil | | | Control | Aqueous | | |
| | | 20 | 40 | 60 | | 20 | 40 | 60 | | 20 | 40 | 60 |
| <i>E. coli</i> O ₁₅₇ :H ₇ | 5 ^e | 7 ^{de} | 9 ^c | 11 ^{bc} | 7 ^{de} | 11 ^{bc} | 13 ^b | 15 ^a | 0 ^f | 7 ^{de} | 7 ^{de} | 8 ^d |
| <i>S. aureus</i> , A.F., 4 | 6 ^f | 9 ^{de} | 11 ^d | 13 ^c | 9 ^{de} | 14 ^c | 17 ^b | 19 ^a | 0 ^g | 7 ^{df} | 8 ^{df} | 9 ^{de} |
| <i>S. typhimurium</i> , A.F., 3 | 5 ^e | 7 ^{de} | 10 ^{bc} | 11 ^b | 7 ^{de} | 10 ^{bc} | 12 ^b | 14 ^a | 0 ^f | 7 ^{de} | 8 ^{cd} | 8 ^{cd} |
| <i>A. niger</i> , H.U.B., 1 | 5 ^e | 7 ^d | 7 ^d | 9 ^b | 5 ^e | 8 ^{cd} | 10 ^b | 14 ^a | 0 ^f | 0 ^f | 0 ^f | 0 ^f |
| <i>A. ochreus</i> , H.U.B., 2 | 5 ^d | 7 ^c | 8 ^{bc} | 9 ^b | 5 ^d | 8 ^{bc} | 9 ^b | 12 ^a | 0 ^e | 0 ^e | 0 ^e | 0 ^e |
| <i>F. oxysporum</i> , H.U.B., 3 | 5 ^g | 8 ^{efc} | 10 ^{bc} | 11 ^b | 6 ^{fg} | 9 ^{bce} | 11 ^b | 15 ^a | 0 ^h | 7 ^{efg} | 8 ^{cef} | 10 ^{bc} |

Values in the same row followed by the same letter (s) do not significantly differ from each other according to Duncan's at 5% level.

Table 3. Inhibition zone (mm) of leek extracts (mg/ml) on different microorganisms.

| Microorganism | Plant extract | | | | | | | | | | | |
|---|----------------|--------------------|-------------------|------------------|------------------|------------------|------------------|-----------------|----------------|-----------------|-------------------|-------------------|
| | Control | Ethanolic | | | Control | Essential oil | | | Control | Aqueous | | |
| | | 20 | 40 | 60 | | 20 | 40 | 60 | | 20 | 40 | 60 |
| <i>E. coli</i> O ₁₅₇ :H ₇ | 5 ^f | 8 ^{de} | 10 ^{cd} | 12 ^{bc} | 7 ^{ef} | 12 ^{bc} | 14 ^b | 16 ^a | 0 ^g | 7 ^{ef} | 9 ^{de} | 10 ^{cd} |
| <i>St. aureus</i> , A.F., 4 | 6 ^h | 10 ^{defg} | 13 ^d | 15 ^c | 9 ^{efg} | 16 ^c | 19 ^b | 22 ^a | 0 ⁱ | 8 ^{fg} | 11 ^{def} | 12 ^{de} |
| <i>S. typhimurium</i> , A.F., 3 | 5 ^f | 9 ^{de} | 11 ^{bcd} | 13 ^{ab} | 7 ^{ef} | 12 ^{bc} | 13 ^{ab} | 15 ^a | 0 ^g | 7 ^{ef} | 7 ^{ef} | 10 ^{bcd} |
| <i>A. niger</i> , H.U.B., 1 | 5 ^e | 8 ^{cd} | 11 ^b | 12 ^b | 5 ^e | 10 ^{bc} | 11 ^b | 14 ^a | 0 ^f | 7 ^{de} | 8 ^{cd} | 10 ^{bc} |
| <i>A. ochreus</i> , H.U.B., 2 | 5 ^e | 7 ^{de} | 9 ^{bcd} | 11 ^{ab} | 5 ^e | 10 ^{bc} | 11 ^{ab} | 13 ^a | 0 ^f | 7 ^{de} | 8 ^{cd} | 10 ^{bc} |
| <i>F. oxysporum</i> , H.U.B., 3 | 5 ^f | 8 ^{de} | 10 ^{cd} | 12 ^{bc} | 6 ^{ef} | 10 ^{cd} | 13 ^b | 15 ^a | 0 ^g | 7 ^{ef} | 8 ^{de} | 10 ^{cd} |

Values in the same row followed by the same letter (s) do not significantly differ from each other according to Duncan's at 5% level.

aqueous extracts and essential oils of onion than *E. coli* O₁₅₇: H₇, *S. aureus*, A.F., 4, *A. niger*, H.U.B., 1, *A. ochreces*, H.U.B., 2 and *F. oxysporum*, H.U.B., No.3 as exhibited by inhibition zones 10, 12, 15, 13, 16 and 18 diameter mm for ethanolic extract and essential oils at different concentrations (20, 40, 60 mg/ml), respectively and 9 and 12 mm for aqueous extract at concentrations (40 and 60 mg/ml, respectively). *F. oxysporum* exhibited inhibition zones 7, 9 and 10 mm for aqueous at concentrations (20, 40 and 60 mg/ml) respectively.

A. niger, H.U.B., 1 was less susceptible than other microorganisms for ethanolic extract and essential oils of onion which exhibited inhibition zones of 7, 9, 10, 8, 10 and 11 mm at concentrations of 20, 40, 60 mg/ml of onion respectively.

All pathogenic bacteria (*E. coli* O₁₅₇: H₇, *Staph aureus*, A.F., 4 and *Salmonella typhimurium*, A.F., 3) were not sensitive to aqueous extract at 20 mg/ml concentration. *A. niger*, H.U.B., 1 and *A. ochreces*, H.U.B., 2 were also unsusceptible to aqueous extract at different concentrations (20, 40, 60 mg/ml). The essential oils at concentration of 60 mg/ml of red onion extracts exhibited significantly higher inhibition zone than other tested extractions.

Inhibition zone (mm) of garlic extracts (mg/ml) on different microorganisms

Data in Table 2 showed that *S. aureus*, A.F., 4 was the most sensitive of the microorganisms to ethanolic extract and essential oils of garlic such exhibiting 9, 11, 13, 14, 17 and 19 mm inhibition zones at the different concentrations (20, 40 and 60 mg/ml) respectively.

Almost all effect of the different concentrations (20, 40 and 60 mg/ml) of garlic aqueous extract on *E. coli* O₁₅₇: H₇, *S. aureus* A.F., 4, *S. typhimurium* A.F., 3 and *F. oxysporum*, H.U.B., 3 were similar. On the other hand, *A. niger*, H.U.B., 1 and *A. ochreces*, H.U.B., 2 were not sensitive to garlic aqueous extract at concentrations (20 and 40 mg/ml). The essential oils at concentration of 60 mg/ml of garlic extracts revealed significantly higher inhibition zone than other tested extractions.

Inhibition zone (mm) of leek extracts (mg/ml) on different microorganisms

Data reported in Table 3 clearly showed that essential oils of leek at 60 mg/ml concentration exhibited higher antimicrobial activity against *S aureus*, A.F., 4 where the inhibition zone reached 22 mm in diameter. On the other hand, the zone of inhibition reached 16, 15, 14, 13 and 15 mm in diameter against of *E. coli* O₁₅₇: H₇, *Salmonella typhimurium*, A.F., 3, *A. niger*, H.U.B., 1, *A. ochreces*, H.U.B., 2 and *F. oxysporum*, H.U.B., 3 respectively. All microorganisms were sensitive to aqueous extract of leek at different concentrations (20, 40 and 60 mg/ml), but 60

Table 4. Effect of essential oils (mg/ml) of onion, garlic and leek on the viable count growth of *E. coli* O₁₅₇: H₇, *S aureus*, A.F., 4 and *Salmonella typhimurium*, A.F., 3 in beef sausage (cfu/g x 10³).

| Microorganism | Essential oils (40 mg/ml) | | |
|---|---------------------------|-----------------|-----------------|
| | Onion | Garlic | Leek |
| <i>E. coli</i> O ₁₅₇ :H ₇ | 80 ^a | 90 ^a | 8 ^b |
| <i>S. aureus</i> , A.F., 4 | 50 ^b | 70 ^a | 4 ^c |
| <i>S. typhimurium</i> , A.F., 3 | 60 ^b | 30 ^a | 20 ^b |

Values in the same row followed by the same letter(s) do not significantly differ from each other according to Duncan's at 5% level; *The used inoculum of *E. coli* O₁₅₇: H₇ was 5 x 10⁸ cfu/ml *The used inoculum of *S. aureus*, A.F., 4 was 6 x 10⁸ cfu/ml; *The used inoculum of *S. typhimurium*, A.F., 3 was 7 x 10⁹ cfu/ml.

mg/ml concentration exhibited high effect against *S. aureus*, A.F., 4 in 22 mm diameter. The maximum inhibition of ethanolic extract was recorded at 60 mg/ml concentration against *E. coli* O₁₅₇: H₇ 12 mm for *S. aureus*, A.F., 4, 15 mm, for *S. typhimurium*, A.F., 3, 13 mm, for *A. niger*, H.U.B., 1, 12 mm, for *A. ochreces*, H.U.B., 2 and 11 mm for *F. oxysporum*, H.U.B., 3 in 12 mm.

Different concentrations (20, 40 and 60 mg/ml) of onion, garlic and leek essential oils were more effective than ethanolic and aqueous extracts against *E. coli* O₁₅₇: H₇, *S. aureus*, A.F., 4, *S. typhimurium*, A.F., 3, *A. niger*, H.U.B., 1, *A. ochreces*, H.U.B., 2 and *F. oxysporum*, H.U.B., 3. Therefore could be used as essential oils for treatment of food to get rid of some pathogenic bacteria which contaminate meat products e.g. *E. coli* O₁₅₇: H₇, *S. aureus* and *S. typhimurium*. The essential oils at concentration of 60 mg/ml of leek extracts had significantly higher inhibition zone than other tested extractions.

Effect of essential oils (mg/ml) of red onion, garlic and leek on survival and growth of *E. coli* O₁₅₇: H₇, *S. aureus* and *S. typhimurium* in beef sausage

The obtained results (Table 4) clearly showed that the concentration of 40 mg/ml of onion essential oils was able to decrease *E. coli* O₁₅₇: H₇, *S. aureus*, A.F., 4 and *S. typhimurium*, A.F., 3 counts in beef sausage from 5x10⁸ to 8x10⁴, 6x10⁸ to 5x10⁴ and 7x10⁸ to 6x10⁴ cfu/g, respectively. Garlic essential oil decreased *E. coli* O₁₅₇: H₇, *S. aureus*, A.F., 4 and *S. typhimurium*, A.F., 3 counts in sausage from 5x10⁸ to 9x10⁴, 6x10⁸ to 7x10⁴ and 7x10⁸ to 3x10⁵ cfu/g, respectively. In addition, leek essential oils revealed higher antimicrobial activity in decreasing of *E. coli* O₁₅₇: H₇, *S. aureus*, A.F., 4 and *S. typhimurium*, A.F., 3 counts in sausage from 5x10⁸ to 8x10³, 6x10⁸ to 4x10³ and 7x10⁸ to 2x10⁴ cfu/g, respectively than other tested essential oils of onion and

Table 5. Inhibition zone (mm) of different concentrations of antibiotics (μg) against different microorganisms.

| Microorganism | Antibiotic (μg) | | | |
|---|------------------------------|-----------------|----------------|-----------------|
| | Oxytetracycline | Tetracycline | Ampicillin | Amoxicillin |
| | 30 | 30 | 10 | 25 |
| <i>E. coli</i> O ₁₅₇ :H ₇ | 25 ^a | 23 ^b | 0 ^d | 17 ^c |
| <i>St. aureus</i> , A.F., 4 | 25 ^a | 20 ^b | 0 ^d | 13 ^c |
| <i>S. typhimurium</i> , A.F., 3 | 30 ^a | 26 ^b | 0 ^c | 25 ^b |
| <i>A. niger</i> , H.U.B.,1 | 0 | 0 | 0 | 0 |
| <i>A. ochreces</i> , H.U.B., 2 | 0 | 0 | 0 | 0 |
| <i>F. oxysporum</i> , H.U.B., 3 | 0 | 0 | 0 | 0 |

Values in the same row followed by the same letter (s) do not significantly differ from each other according to Duncan's at 5% level.

leek. It could be concluded that the essential oils of leek showed significantly pronounced effect on the reduction of all counts of tested microorganisms.

The extent of the inhibitory effect of the onion extracts could be attributed to the presence of antimicrobial compounds and their dissolving ratios in the solvents and concentration doses. The same observations were reported by Jeyakumar et al. (2005) who reported the bacterial effect of onion extracts against *E. coli*, *S. aureus* and *S. enteritidis* by using agar diffusion method. Srinivasan et al. (2001) reported moderate antibacterial activity of an onion extract against *E. coli* and *S. typhimurium*. Indu et al. (2006) demonstrated that the various concentrations of an onion extract field inhibited the growth of *S. enteritidis* and *S. typhimurium*. Good antibacterial activity of an onion extract on the growth of *S. enteritidis* was also reported by Suresh et al. (2006). On the other hand, Elnima et al. (1983) reported that 66% of aqueous extracts of red onion inhibited the growth of *S. aureus*. In other families, the ethanol extract showed more activity than the water extracts perhaps due to the increased solubility of the active principle in ethanol (Vaijayanthimala et al., 2000). The major antimicrobial compound in garlic is allicin (Conner, 1993). Garlic extracts have been found to possess antibacterial property against *S. typhimurium*, *E. coli* No.1, *Staphylococcus epidermidis* and *Staphylococcus aureus* (Arora and Kaur, 1999). The inhibitory activity of EO extracts of *Allium* plants against mold was reported by Zaika (1988). Garlic showed highest antifungal activity against three *Aspergillus* species tested (Yin and Tsao, 1997).

Benkeblia (2004) stated that *F. oxysporum* showed the lowest sensitivity towards EO extracts of garlic and onion whereas *A. niger* No.1 was significantly inhibited particularly at low concentrations. Bandna (2013) mentioned that garlic extracts exhibited excellent antibacterial activity against *E. coli*, *S. typhi* and *S. aureus*. In contrast, water extracts of garlic were reported to be more potent than ethanol and chloroform extracts against the tested microbes in the study of Abubakar

(2009). The ethanolic extract of garlic was more effective than the aqueous extract, inhibiting all the test organisms. While the aqueous extract was effective against *E. coli*, *P. aeruginosa* and *Klebsiella pneumonia* (Arekemase et al., 2013). Ethanolic extract of garlic significantly inhibited growth of *A. flavus* and *A. niger* (Onyeagba et al., 2004). Dankert et al. (1979) reported that garlic extracts were very effective in inhibiting the growth of *Aspergillus* species. As observed by Naem-Rana and Hadi-Noora (2012), aqueous extract of *Aporrum* (leek) had antimicrobial activity against *S. aureus*, *P. aeruginosa* and *E. coli*. Benkeblia et al. (2005) showed that leek inhibited the growth of *E. coli* and *S. aureus*. As explained by Breu and Dorsch (1994) aqueous and alcoholic extracts of leek leaves have a powerful antibacterial activity as they inhibit the growth of *E. coli*, *S. aureus*, *S. marcescens*, *P. aeruginosa* and *S. typhi*.

Inhibition zone (mm) different concentrations of antibiotics (μg) against different microorganisms

Data recorded in Table 5 revealed that *S. typhimurium*, A.F.,3 was more susceptible than *E. coli* O₁₅₇:H₇ and *S. aureus*, A.F.,4 against oxytetracycline 30 μg , tetracycline 30 μg and amoxicillin 25 μg which exhibited inhibition zone diameter of 30, 26 and 25 mm for oxytetracycline 30 μg , tetracycline 30 μg and amoxicillin 25 μg , respectively, while both *E. coli* O₁₅₇:H₇, *S. aureus*, A.F.,4 and *S. typhimurium*, A.F.,3 were not sensitive to ampicillin 10 μg . On the other hand, both *A. niger*, H.U.B.,1, *A. ochreces*, H.U.B.,2 and *F. oxysporum*, H.U.B., 3 were unsusceptible to oxytetracycline 30 μg , tetracycline 30 μg , ampicillin 10 μg and amoxicillin 25 μg .

The antimicrobial activity of ethanolic and aqueous extracts and essential oils of red onion, garlic and leek were compared with that of antibiotics (oxytetracycline, tetracycline, ampicillin and amoxicillin) which is considered to be accepted in the treatment of diseases caused by types of standard bacteria and fungi species. Ampicillin is not appropriate for infections caused by *E.*

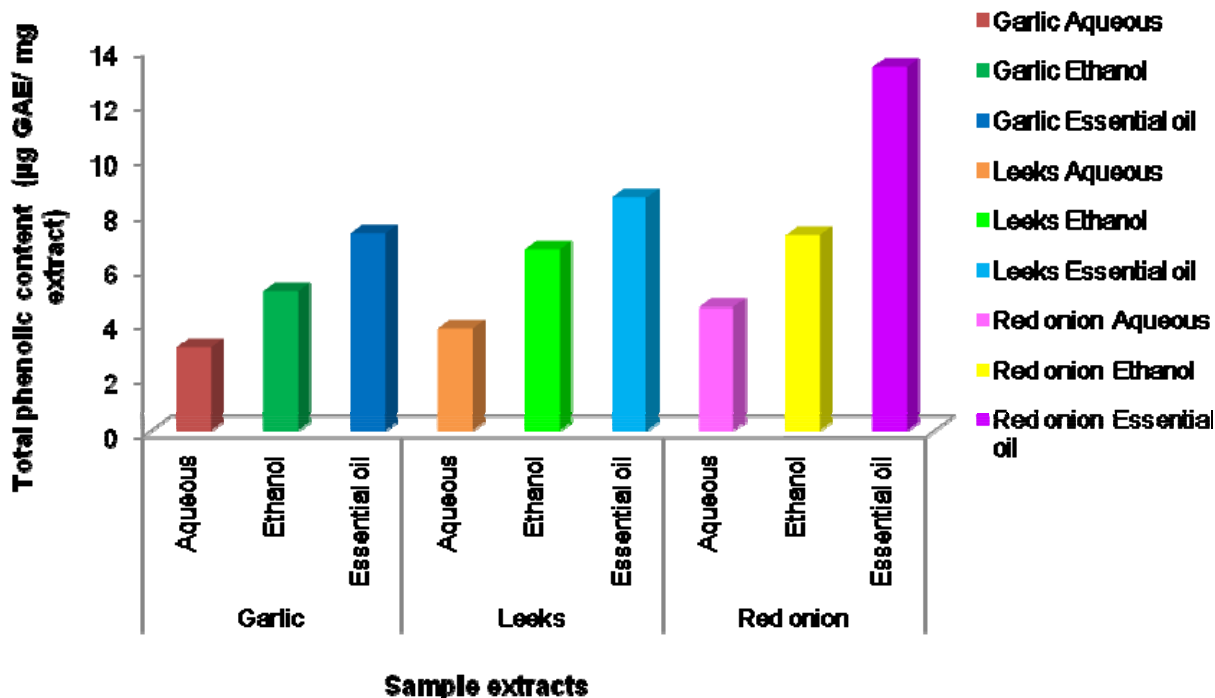


Figure 1. Total phenolic contents of the essential oils, aqueous and ethanolic extracts of red onion, garlic and leek.

*coli O*₁₅₇:H₇, *S. aureus*, A.F.,4, *S. typhimurium*, A.F., 3, *A. niger*, H.U.B.,1, *A. ochreus*, H.U.B.,2 and *F. oxysporum*, H.U.B.,3, but oxytetracycline, tetracycline and amoxicillin can be used as a broad spectrum drug against these pathogenic bacteria, such can be substituted for natural antimicrobial agents. Oxytetracycline showed significantly higher inhibition zone than other tested antibiotics.

Total phenolic content

Total phenolic contents of the essential oils, aqueous and ethanolic extracts of red onion, garlic and leek are presented in Figure 1. In general, the total phenolic contents were higher in essential oils extracts of red onion, garlic and leek whereas the lower contents were shown in aqueous extracts. The highest level of phenolic contents of red onion, garlic and leek were found in red onion (13.34 µg GAE/ mg extract), while the lowest contents were found in garlic (7.25 µg GAE/ mg extract). A similar observation has been reported by Gorinstein et al. (2008) who found that the total concentration of phenolic was higher in red onion than in white onion and in garlic.

DPPH radical scavenging activity

DPPH is a free radical compound that has been widely used to determine the free radical scavenging capacity of

various samples because of its stability (in radical form), simplicity and fast assay. The results of DPPH free radical-scavenging ability of the essential oils aqueous and ethanolic extracts of red onion, garlic and leek are shown in Figure 2.

The present investigation depicts that the essential oils extracts have more stronger antioxidant properties than ethanol and aqueous extracts of red onion, garlic and leek used in the present study. The essential oils extracts of red onion had the strongest radical-scavenging effect (30.81%), while the essential oils extracts of garlic had the lowest radical-scavenging effect (22.04 %). These findings are in agreement with the data of Lee et al. (2012) who showed that red onion exhibited approximately six and fivefold higher DPPH radical-scavenging activity than garlic and white onion, respectively.

Conclusion

From this study, it could be recommended to apply onion, garlic and leek in controlling infection by *E. coli O*₁₅₇:H₇, *S. aureus*, A.F.,4, *S. typhimurium*, A.F.,3, *A. niger*, H.U.B.,1, *A. ochreus*, H.U.B.,2 and *F. oxysporum*, H.U.B.,3, to prevent cold meat spoilage and preserve meat for longer periods against pathogenic bacteria mentioned above. The three extracts from *Allium* common plants which had higher phenolic content showed higher antioxidant activity in terms of DPPH

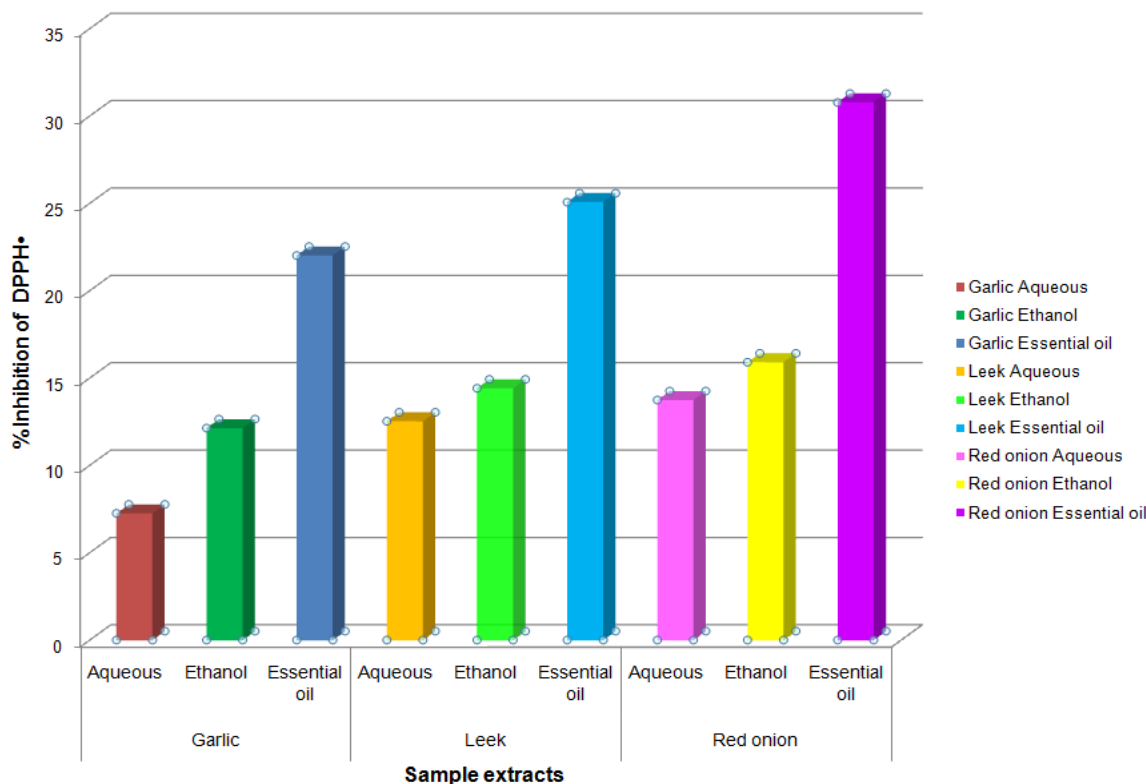


Figure 2. Inhibition effects of the essential oils, aqueous and ethanolic extracts of red onion, garlic and leek DPPH radical.

radical scavenging activity. The phenolic contents could be used as an important indicator of the antioxidant capacities.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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