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# Antimicrobial properties of 39 essential oils against thirteen food-borne microorganisms; efficacy and environmental hygiene of *Prunus armeniaca* in raw food preservation under cold storage

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## ABSTRACT

**Aim:** The present study investigated the antimicrobial properties of 39 diversified essential plant oils (EOs). The most bioactive EO was selected and tested for its environmental hygiene efficacy in the preservation of stored raw food. **Methods:** The antimicrobial efficacy of 39 EOs was examined against 13 representative food-borne microorganisms. Minimal inhibitory concentration (MIC) of extracted apricot (*Prunus armeniaca*) seed EO was evaluated. Different concentrations of extracted oil were applied to four types of low-fat raw foods under cold dry storage. **Results:** The results of the microbial sensitivity assay showed considerable positive responses to only 23 out of 39 EOs. *P. armeniaca* exhibited the most significant antimicrobial efficacy. Different MIC values of extracted *P. armeniaca* oil were documented as a result of strain variability of representative food-borne microorganisms. Extracted apricot EO concentration delayed bacterial food spoilage at 1000 $\mu$g/ml while fungal spoilage delayed at 2000 $\mu$g/ml. Total bacterial viable count (TVC) of raw food samples treated with 1000 $\mu$g/ml oil decreased sharply when compared with TVC of samples not treated with oil. Fungal growth was completely inhibited in samples treated with 2000 $\mu$g/ml oil. Statistical analysis showed a significant association between the MIC of *P. armeniaca* EO and the growth of the 13 representative food-borne microorganisms, it was mostly 500 $\mu$g/ml. **Conclusion:** The achieved study results support using of *P. armeniaca* EO in controlling shelf-life of raw foods stored under dry cold conditions.

KEY WORDS: Antimicrobial efficacy, dry cold storage, essential oils, food-borne microorganisms, minimal inhibitory concentration, *Prunus armeniaca*, raw foods

## INTRODUCTION

Food safety is one of the most fundamental public health issues due to spreading of food-borne illness outbreaks annually caused by some pathogens and/or their enterotoxins [1]. Food-borne diseases can also be caused by other types of microbial toxins, such as by the neurotoxins produced by *Clostridium botulinum*. Moreover, microbial growth and metabolism are associated with food spoilage, causing economic losses [2].

As refrigeration alone cannot maintain food quality during storage, food preserved with naturally derived antimicrobial ingredients is being popularly used due to increase of consumer perception and concern regarding synthetic chemical additives [3].

Plant-derived essential oils (EOs) have been recognized as flavoring agents in foods. Many of these EOs have high antimicrobial efficacies against food-borne microorganisms. This property enables EOs to be applied as natural agents in food preservation, so sustain the shelf life of processed foods [4].

The impact of EOs on organoleptic acceptability of foods is an important aspect to optimize application of them in food.

Unacceptable levels of flavors and odors may result from addition of high concentrations of EOs to achieve adequate antimicrobial activity [2].

Therefore, the current study was designed to evaluate antimicrobial efficacy of 39 diverse plant EOs against 13 representative food-borne bacteria and fungi. Also, it aimed to determine minimal inhibitory concentration (MIC) of *Prunus armeniaca* oil and assess its efficacy and environmental hygiene in microbial food spoilage biocontrol. *P. armeniaca* seed EO was selected to study as it had been proven to have the highest biocontrol efficacy and the minimum effect in food taste (organo-found leptical criteria). Moreover, all previous scientific studies investigated the antimicrobial efficacy and environmental hygiene of EOs did not include apricot seed EO.

## MATERIALS AND METHODS

### Study Design

The study was performed on 39 miscellaneous commercial EOs, to examine their inhibitory activity against 13 representative food-borne microorganisms of economic and hygienic significance. EO extracted from apricot (*P. armeniaca*) seeds was selected to be investigated for its effectiveness in raw food shelf-life extending. Evaluation of food total viable count (TVC) and fungal food spoilage were the indicators of EO efficacy.

### EO, Plant Material and Raw Food

39 miscellaneous commercial plant EOs were collected from 2 brands, "Al-ahlam for seeds oil" (Production Jeddah, Saudi Arabia) and "Al Captain Company" (Cairo, Egypt). The 39 EOs were *Thyme vulgaris*, *Nigella sativa*, *P. armeniaca*, *Eruca sativa*, *Curcume longa*, *Prunus amygdoles*, *Ricinus communis*, *Olea europaea*, *Allium sativum*, *Allium cepa*, *Foeniculum vulgare*, *Syzgium aromaticum*, *Linum usitatissimum*, *Helianthus annus*, *Zingiber officinale*, *Cinnamon vulgare*, *Aloe vera barbadensis*, *Sesamum inicum*, *Mentha piperita*, *Lupinus termis*, *Anthemic nobilis*, *Ocimum basilicum*, *Sinapis alba*, *Eucalyptus sp.*, *Pistacia lentiscus*, *Myristica fragrans*, *Cuminum cyminum*, *Pimpinella anisum*, *Panax ginseng*, *Carium carvi*, *Elettaria cardamomum*, *Rosmarinus officinalis*, *Origanum vulgare*, *Petroselinum crispum*, *Crocus sativus*, *Citrus sinensis*, *Armoracia rusticane*, *Coriandrum sativum* and *Trigonella foenum*. Ripe seeds of *P. armeniaca* and raw food samples: minced meat, skinned poultry, wheat grains and whole tomato fruits were purchased from the local markets in Alkharj (Kingdom of Saudi Arabia) and Giza (Egypt) cities. Samples of EOs, apricot seeds and wheat grains were collected and preserved in refrigerator under dry condition (refrigerator of no frost property) for the bioassay study. Other fresh foods were immediately undergone bioassay.

### Microorganisms

13 representative food-borne microbial strains (9 bacterial and 4 fungal species) were selected, due to their relevance in food industry, economy and medical aspects; *Staphylococcus aureus* ATCC 6538, *Clostridium perfringens* CPE str. F4969, *Bacillus cereus* CECT 495, *Lactobacillus brevis* ATCC 10876, *Bacillus stearothermophilus* DSM 297, *Salmonella typhimurium* DSM 5569, *Escherichia coli* O157:H7 ATCC 43888, *Enterobacter aerogenes* KCTC 2190, *Pseudomonas aeruginosa* KCTC 2004, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium lycopersici* and *Alternaria solani*.

All fungal and bacterial strains were obtained from Botany Department, Faculty of Women for Arts, Science and Education, Ain Shams Univ., Egypt. Microbial cultures were maintained on their appropriate agar media at 4°C to be used as stock cultures. Bacteria were maintained on nutrient agar medium (NA), while fungi on sabouraud chloramphnicol Agar (SA) (2.0 mg chloramphnicol/1 ml of Sabouraud agar medium).

### Antimicrobial - Susceptibility Testing

Antimicrobial assay of all tested 39 EOs was based on agar diffusion method [5] by applying Agar-well diffusion test [2]. Antifungal evaluation was according to Ibrahim [6], while bacteria to Bajpai *et al*. [7].

In antibacterial assay; each bacterial strain was pre-cultured on Mueller Hinton broth over night under its corresponding conditions of incubation. All bacterial strains were incubated under aerobic conditions for 24 h at 37°C except *C. perfringens* CPE str. F4969 and *L. brevis* ATCC 10876 were incubated under anaerobic conditions using anaerobic jar and *B. stearothermophilus* DSM 297 was incubated at 53-55°C. Strain broth culture was adjusted to final density of $10^6$ CFU/ml using the McFarland standard (Biomerieux Inc.), and used as inoculum. A Petri dish containing sterile NA was seeded with 100 $\mu$l inoculum of the target bacteria. Wells of 1.0 cm diameter were aseptically bored into NA center and subsequently 1.0 ml of the tested EO was added in each well. Negative control was prepared using 1.0 ml of sterile saline solution instead of oil. 1 ml of standard reference antibiotic chloramphnicol (2.0 mg chloramphnicol per one ml sterile water) was used as positive control. The plates were kept at 4°C for 2-3 h to allow diffusion of each substance into the agar then incubated for 24 h under opportune conditions of strain incubation mentioned up. Antibacterial activity was evaluated by measuring the diameter of inhibition zones surrounding the wells using a Vernier-Caliper then samples were compared with its positive and negative controls.

In antifungal assay; Petri dishes containing sterile SA were seeded with 1.0 ml of test EO per plate. An SA disc of 1.0 cm diameter, pre-cultivated with target fungal strain for 7 days at 28°C, was transferred as an inoculum to the surface of the sterile SA on the center. All plates were incubated at 28°C for 7 days. Negative control was prepared using SA seeded with 1.0 ml sterile saline solution; while in positive control, SA was seeded with 1.0 ml standard reference antibiotic Mycostatine (2.0 mg Mycostatine per ml) per plate. Antifungal activity was detected on macroscopic evaluation of fungal growth density.

Fungal growth density of samples was compared each with its corresponding positive and negative controls then scored as specified by Ibrahim [6]. The Scores of +++ were graded as good growth, ++ as moderate growth, + as weak growth and scores of - were graded as completely inhibited.

### Extraction of *P. armeniaca* EO

The external hard covers of apricot seeds (*P. armeniaca*) were eliminated and the seeds were dried for 2 weeks then pulverized using a super mixed blender into powder form. The powder (200 g) was subjected to hydro-distillation for 3 h using a Clevenger - type apparatus. The oil was dried over anhydrous $Na_2SO_4$ and preserved in a sealed vial at 4°C prior to further studies.

### Determination of MIC

MIC of the extracted apricot oil was tested by a two-fold serial dilution method [8]. To prepare stock solution of oil sample, 1.0 g extracted apricot EO was dissolved in 10 ml 5% dichloromethane. For bacterial assay, oil stock solution was added to sterile Mueller Hinton broth to get a final concentration of 4000 $\mu$g/ml (10 ml stock/36.25 ml broth medium), which was further serially diluted using double strength broth medium to achieve 2000, 1000, 500, 250, 125 $\mu$g/ml respectively. Thereafter, all dilutions were dispersed into tubes (10.0 ml broth/one tube). 10 $\mu$l of standardized bacterial suspension ($10^6$ CFU/ml) was used as inoculum per tube. Negative control, oil-free broth, was also prepared for each strain. All tubes together with control were subsequently incubated for 24 h under strain corresponding incubation conditions.

For fungal assay, same extracted oil concentrations were prepared using double strength sterile SA then dispersed into sterile Petri dishes. An SA disc of 1 cm pre-cultivated fungal strain was used as an inoculum. Negative control was also prepared. All Petri dishes were incubated at 28°C for 7 days.

The lowest concentration of extracted *P. armeniaca* EO sample, which did not show any growth of test organisms after macroscopic evaluation, was determined as MIC, which was expressed in $\mu$g/ml.

### Effect of EO Extracted *P. armeniaca* on the Spoilage of Raw Foods Stored Under Two Different Storage Conditions

Fresh raw food samples (skinned poultry, minced meat, wheat grains and whole tomato fruits) were collected fortnightly, one sample of each food type was collected per 2 weeks i.e., two samples every month, for a period of 6 months, giving a total of 12 samples for each food. Samples were collected, preserved and examined biologically according to the Laboratory Methods in Food Microbiology [9]. Oil antimicrobial bioassay was conducted for evaluation of TVC for antibacterial assay and detection of fungal food spoilage for antifungal assay. Tomato fruits were well cleaned, washed and scratched then inoculated by *A. solani*. Wheat grains were sprayed with sterile water then inoculated by both *A. flavus* and *A. niger* (one loop full fungal spore of each strain/100 g grains) and mixed well. Two sets of food samples were prepared. Each set was composed of oil both treated and not treated skinned poultry, minced meat and previously fungal inoculated wheat grains and tomato fruits samples. Oil treated food samples were samples sprayed with *P. armeniaca* EO (1.0 ml EO/100 g food sample and 1.0 ml EO/one tomato fruit) of 2000, 1000, 500, 250 and 125 $\mu$g/ml. Oil not treated food samples (negative control) were samples not sprayed with oil (oil-free). Then, one set of samples was stored under dry refrigeration (at 4°C), and the other set at room temperature (at 25°C). Data of TVC evaluation in skinned poultry and minced meat was collected daily as same as detection of fungal spoilage on wheat grains and whole tomato fruits. According to the Egyptian [10,11] and Saudi standards [12] compared with recorded data, percentages of acceptable and unacceptable environmental hygienic food samples were determined.

### Statistical Analysis

Data were tabulated and analyzed using Statistical Package for Social Sciences, version 11.0 computer software package [13].

## RESULTS

### EOs Antimicrobial Efficacy

On comparing recorded data with negative controls, it was found that the 13 representative food-borne microorganisms were positively sensitive to 23 EOs out of 39 tested EOs. Table 1 demonstrated the antimicrobial properties of these 23 bioactive oils. 21 EOs out of the 23 EOs showed antibacterial response, while only 15 EOs was antifungal bioactive as shown in Table 1.

On the other hand, 16 EOs out of tested 39 EOs exhibited no antimicrobial potential; These 16 EOs were *E. sativa*, *P. amygdoles*, *R. communis*, *F. vulgare*, *A. cepa*, *F. vulgare*, *L. usitatissimum*, *H. annus*, *S. inicum*, *L. termis*, *A. nobilis*, *P. lentiscus*, *M. fragrans*, *P. ginseng*, *C. carvi*, *C. sativus*. Microbial susceptibility to EOs showed that oils with the highest inhibitory effects produced bacterial inhibition zones of 17-22 mm diameter, while resulted in completely or strong inhibition of fungal growth. Variable responses of bacterial strain sensitivity were detected against only 21 EOs as demonstrated in Table 1. In general, the representative food-borne Gram-negative bacteria and *B. stearothermophilus* were more resistant to the antibacterial properties of EOs than Gram-positive bacteria. The widest bacterial inhibition spectrum appeared with EO extracted from *P. armeniaca* which showed bacterial inhibition zones ranging from 14 to 22 mm followed by *T. vulgaris*, *C. vulgare*, *S. aromaticum* and *O. vulgare*. On contrast, *R. officinalis*, *A. barbadensis*, *O. basilicum* and *P. crispum* exhibited the lowest antibacterial activity with 11-12 mm inhibition zones or even no detectable zones.

Regarding the developing a new highly active antifungal food preservative, antifungal properties of 39 commercial EOs against four species of representative food-borne *filamentous*

Table 1: Bioactivity of commercial essential oils effective against food-borne pathogenic and spoilage test microorganisms

| Microbial strain | Inhibition zone diameter (mm) | | | | | | | | | Fungal growth | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Active essential oils | *S. aureus* | *C. perfringens* | *B. cerus* | *S. typhimurium* | *E. coli* 0157: H7 | *L. brevis* | *B. stearothermophilus* | *E. aerogenes* | *P. aeruginosa* | *A. niger* | *A. flavus* | *F. lycopersici* | *A. solani* |
| *T. vulgaris* | 20 | 19 | 18 | 15 | 16 | 18 | 13 | 14 | 15 | - | - | - | - |
| *P. armeniaca* | 22 | 20 | 20 | 17 | 17 | 20 | 14 | 16 | 17 | - | - | - | - |
| *C. sinensis* | 16 | 15 | 16 | 14 | 13 | 15 | 12 | 13 | 14 | +++ | +++ | +++ | +++ |
| *O. vulgare* | 18 | 16 | 16 | 14 | 14 | 16 | ND | 15 | 14 | + | + | ++ | + |
| *T. foenum* | 12 | 13 | 12 | ND | ND | 13 | ND | 12 | 13 | +++ | +++ | +++ | +++ |
| *A. rusticane* | 14 | 13 | 13 | 12 | 12 | 13 | ND | 11 | 12 | ++ | ++ | ++ | ++ |
| *C. longa* | 13 | 12 | 13 | 11 | 11 | 14 | ND | 12 | 12 | +++ | +++ | ++ | ++ |
| *A. sativum* | 14 | 12 | 12 | 11 | 11 | 12 | ND | 12 | 11 | ++ | ++ | + | + |
| *S. aromaticum* | 19 | 16 | 16 | 14 | 14 | 16 | 13 | 14 | 14 | ++ | ++ | ++ | ++ |
| *S. alba* | 13 | 13 | 13 | 12 | 12 | 13 | 12 | 13 | 13 | + | ++ | + | + |
| *C. vulgare* | 19 | 17 | 18 | 14 | 15 | 17 | 13 | 15 | 15 | + | + | + | + |
| *C. cyminum* | 16 | 15 | 15 | 13 | 13 | 16 | ND | 13 | 13 | +++ | +++ | +++ | +++ |
| *P. anisum* | 14 | 13 | 14 | 12 | 12 | 12 | ND | 12 | 12 | +++ | +++ | +++ | +++ |
| *A. barbadensis* | 12 | 11 | 11 | 11 | 11 | 12 | ND | 11 | 11 | ++ | ++ | ++ | ++ |
| *Z. officinale* | 17 | 15 | 15 | 13 | 13 | 15 | 13 | 13 | 13 | +++ | +++ | +++ | +++ |
| *E. cardamomum* | 13 | 12 | 12 | ND | ND | 12 | ND | 11 | 11 | +++ | +++ | +++ | +++ |
| *R. officinalis* | 12 | ND | ND | 11 | 11 | ND | ND | 12 | 11 | +++ | +++ | +++ | +++ |
| *C. sativum* | 14 | 15 | 16 | 12 | 12 | 15 | ND | 12 | 11 | +++ | +++ | +++ | +++ |
| *N. sativa* | 13 | 14 | 14 | 12 | 12 | 13 | 12 | 13 | 12 | ++ | ++ | ++ | ++ |
| *O. europaea* | ND | ND | ND | ND | ND | ND | ND | ND | ND | - | - | - | - |
| *M. piperita* | ND | ND | ND | ND | ND | ND | ND | ND | ND | - | - | - | - |
| *O. basilicum* | 11 | 11 | ND | 11 | ND | ND | ND | 12 | ND | +++ | +++ | ++ | ++ |
| *P. crispum* | 12 | 12 | 11 | ND | 11 | 12 | ND | ND | ND | +++ | +++ | ++ | ++ |

ND: Not detected, +++: Good growth, ++: Moderate growth, +: Weak growth, - : Completely inhibited, *S. aureus: Staphylococcus aureus, C. perfringens: Clostridium perfringens, B. cerus: Bacillus cerus, E. aerogenes: Enterobacter aerogenes, P. aeruginosa: Pseudomonas aeruginosa, A. niger: Aspergillus niger, A. flavus: Aspergillus flavus, F. lycopersici: Fusarium lycopersici, A. solani: Alternaria solani, T. vulgaris: Thyme vulgaris, P. armeniaca: Prunus armeniaca, C. sinensis: Citrus sinensis, O. vulgare: Origanum vulgare, T. foenum: Trigonella foenum, A. rusticane: Armoracia rusticane, C. longa: Curcume longa, A. sativum: Allium sativum, S. aromaticum: Syzgium aromaticum, S. alba: Sinapis alba, C. vulgare: Cinnamon vulgare, C. cyminum: Cuminum cyminum, P. anisum: Pimpinella anisum, A. barbadensis: Aloe vera barbadensis, Z. officinale: Zingiber officinale, E. cardamomum: Elettaria cardamomum, R. officinalis: Rosmarinus officinalis, C. sativum: Coriandrum sativum, O. europaea: Olea europaea, M. piperita: Mentha piperita, O. basilicum: Ocimum basilicum, P. crispum: Petroselinum crispum, N. sativa: Nigella sativa, S. typhimurium: Salmonella typhimurium, E. coli 0157: H7: Escherichia coli 0157: H7, L. brevis: Lactobacillus brevis, B. stearothermophilus: Bacillus stearothermophilus*

*fungi* were assayed. It is clear from Table 1 that fungal growth, as compared with negative control, was affected variably with only 15 out of 23 bioactive EOs. Tested spices as *S. alba*, *O. vulgare* and *C. vulgare* showed antifungal efficacies generally more than antibacterial. Fungal growth was inhibited strongly emerging weak growth. Completely inhibition of fungal growth was observed with *P. armeniaca*, *T. vulgaris*, *O. europaea* and *M. piperita*. However, *C. longa* followed by *P. crispum* and *O. basilicum* were the weakest EO against fungi.

## MIC of Extracted *P. armeniaca* EO

In the dose response study, documented data were compared with negative controls and it was found that microbial growth inhibition increased with increasing *P. armeniaca* EO concentration. Low EO concentration (125 μg/ml) weakly inhibited the growth of all bacterial strains except *S. aureus*, the growth was markedly very weak. Growth of all representative food-borne Gram-negative bacteria was inhibited at 250 μg/ml while fungal growth did not affect. On the other hand, at high concentration (500 μg/ml), the EO exhibited a marked growth inhibition against *F. lycopersici* and *A. solani* and completely inhibited all representative food-borne bacteria except *B. stearothermophilus*. A concentration of 1000 μg/ml was highly active against the growth of *B. stearothermophilus*, *A. niger* and *A. flavus*. As a result of achieved data, it was found that 125 μg/ml was the *P. armeniaca* EO MIC against *S. aureus*, while 250 μg/ml was the MIC against all representative food-borne Gram-negative bacteria. *P. armeniaca* EO MIC against *F. lycopersici*, *A. solani* and other mesophilic Gram-positive bacteria was established at 500 μg/ml. Moreover, MIC at 1000 μg/ml was documented with *B. stearothermophilus*, *A. niger* and *A. flavus*.

Spearman rank correlation was used to correlate between MIC values of *P. armeniaca* EO and the growth of representative food-borne microorganisms. Results of statistical analysis summarized in Table 2 showed a negative correlation between microbial growth and MIC value of *P. armeniaca* EO; where the more MIC value, the less microbial growth. This negative correlation assessed in this study indicated that MIC of *P. armeniaca* EO is suitable in the food safety and quality control.

## Food Spoilage Biocontrol Effectiveness of *P. armeniaca* EO

*P. armeniaca* EO was tested for its ability to be a natural food preservative against food spoilage microorganisms. All used samples were low fat foods; on assayed, it were effectively affected in oil biocontrol activity. As a result, antimicrobial efficacy of EOs was affected by food system composition.

According to estimated data, the best antibacterial efficacy of extracted *P. armeniaca* EO was detected at concentration 1000 μg/ml, where TVC was markedly declined when was compared with negative control TVC. While on comparing fungal food spoilage of oil treated tomato and wheat grains with oil-free samples, it was found that at oil concentration 500 μg/ml, best antifungal EO efficacy was noticed in tomato samples, where symptoms of fungal spoilage of were obviously delayed. However, fungal spoilage symptoms of wheat grain samples were clearly delayed at oil concentration 2000 μg/ml.

TVC evaluation in negative controls of oil-free skinned poultry and minced meat samples exhibited very high count after storage period of 5-8 days at 4°C. However, in room temperature storage, very high TVC was evaluated after 1 day for minced meat and 2 days for poultry.

Documented data, illustrated in Figure 1, indicated that refrigerated food samples treated with the EO extracted from *P. armeniaca* seeds and stored under dry conditions showed a higher tendency to decrease TVC and delay fungal growth than in oil-free samples stored at room temperature. In oil treated samples stored at 4°C under dry conditions for 14 days, TVC decreased in 83.3% samples of minced meat and in 91.7% skinned poultry samples on comparing with their corresponding oil-free samples. Whereas, TVC were not affected in 16.7% minced meat and 8.3% poultry samples.

When oil treated samples were stored at 25°C (unrefrigerated) for 14 days, TVC percentage was not affected in 41.7% minced meat and 33.3% skinned poultry samples as compared with oil-free samples. While it decreased in 58.3% minced meat and in 66.7% of skinned poultry samples as demonstrated in Figure 1.

Table 3 demonstrated that the majority (83.3%) of oil treated tomato samples (10 out of 12 samples) stored under dry conditions at 4°C showed fungal spoilage within 23 days, while spoilage appeared in 16.7% of samples after 20 days (2 samples). Moreover, fungal spoilage emerged in three quarter percentage (75.0%) of oil treated wheat grain samples (9 out of 12 samples) stored at 4°C under dry conditions after 90 days, while emerged after 95 days in 25.0% samples (3 samples). When samples were stored at 25°C, more than half percentage (66.7% and 58.3%) of oil treated tomato and wheat samples (8 and 7 samples) exhibited fungal spoilage after 15 and 50 days respectively. In addition, more than third percentage (33.3% and 41.7%) of tomato and wheat samples (4 and 5 samples) exhibited fungal spoilage after 13 and 55 days respectively.

Fungal spoilage of negative controls (oil-free) in both tomato and wheat samples were detected after storage period of 8

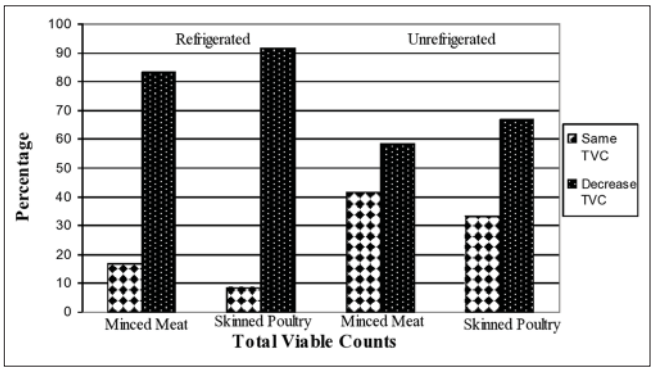


**Figure 1:** Total viable counts of the examined minced meat and skinned poultry samples under *Prunus armeniace* biocontrol according to storage temperature for 14 days

Table 2: Correlation between MICs of *P. armeniaca* essential oil and growth of food-borne microorganisms

| MICs of *P. armeniaca* oil | Bacteria | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Gram-positive | | | | | | | | | |
| | *S. aureus* | | *C. perfringens* | | *B. stearothermophilus* | | *B. cerus* | | *L. brevis* | |
| | Correlation coefficient | Sig. (2-tailed) | Correlation coefficient | Sig. (2-tailed) | Correlation coefficient | Sig. (2-tailed) | Correlation coefficient | Sig. (2-tailed) | Correlation coefficient | Sig. (2-tailed) |
| 125 | −10.125 | 0.000* | −9.988 | 0.006 | −21.051 | 0.009 | −9.575 | 0.004 | −17.181 | 0.005 |
| 250 | −15.321 | 0.001 | −5.988 | 0.007 | −15.142 | 0.006 | −24.873 | 0.006 | −14.755 | 0.002 |
| 500 | −20.116 | 0.005 | −2.868 | 0.000*** | −12.131 | 0.001 | −5.325 | 0.000** | −13.208 | 0 |
| 1000 | −22.865 | 0.008 | −3.868 | 0.001 | −11.092 | 0 | −7.291 | 0.001 | −10.25 | 0.002 |
| MICs of *P. armeniaca* oil | Bacteria | | | | | | | | | |
| | Gram-negative | | | | | | | | | |
| | *S. typhimurium* | | *E. coli* 0157:H7 | | *E aerogenes* | | *P. aeruginosa* | | | |
| | Correlation coefficient | Sig. (2-tailed) | Correlation coefficient | Sig. (2-tailed) | Correlation coefficient | Sig. (2-tailed) | Correlation coefficient | Sig. (2-tailed) | | |
| 125 | −9.279 | 0.002 | −9.42 | 0.104 | −6.235 | 0.669 | −4.873 | 0.083 | | |
| 250 | −5.391 | 0 | −2.143 | 0 | −8.069 | 0.515 | −3.452 | 0.024 | | |
| 500 | −8.609 | 0.102 | −8.67 | 0.012 | −4.32 | 0 | −1.763 | 0.000** | | |
| 1000 | −10.25 | 0.17 | −6.341 | 0.061 | −2.687 | 0.34 | −2.001 | 0.003 | | |
| MICs of *P. armeniaca* oil | Fungi | | | | | | | | | |
| | *A. niger* | | *A. flavus* | | *F. lycopersici* | | *A. solani* | | | |
| | Correlation coefficient | Sig. (2-tailed) | Correlation coefficient | Sig. (2-tailed) | Correlation coefficient | Sig. (2-tailed) | Correlation coefficient | Sig. (2-tailed) | | |
| 125 | | | | | | | | | | |
| 250 | −9.483 | 0.32 | −11.97 | 0.1 | −8.42 | 0.005 | −22.301 | 0.007 | | |
| 500 | −6.157 | 0.201 | −12.768 | 0.5 | −3.01 | 0 | −14.951 | 0 | | |
| 1000 | −1.92 | 0.000* | −10.768 | 0.000** | −5.548 | 0.001 | −10.153 | 0.001 | | |

*Correlation is significant at 0.05 level, **Correlation is significant at 0.01 level,***Correlation is significant at 0.001 level. *P. armeniaca: Prunus armeniaca, S. aureus: Staphylococcus aureus, C. perfringens: Clostridium perfringens, B. stearothermophilus: Bacillus stearothermophilus, B. cerus: Bacillus cerus, L. brevis: Lactobacillus brevis, S. typhimurium: Salmonella typhimurium, E. coli: Escherichia coli, E. aerogenes: Enterobacter aerogenes, P. aeruginosa: Pseudomonas aeruginosa, A. niger: Aspergillus niger, A. flavus: Aspergillus flavus, F. lycopersici: Fusarium lycopersici, A. solani: Alternaria solani*, Sig: Significant, MIC: Minimal inhibitory concentration

and 30 days respectively at 4°C. However, regarding room temperature storage, fungal spoilage was detected after 5 days for tomato and 17 days for wheat samples.

A significant fact was achieved on examining the preservative efficacy of *P. armeniaca* EO on raw foods against microbial food spoilage during storage, *P. armeniaca* EO was able to prolong shelf life time of foods and reduced the survival of spoilage microorganisms.

In estimating TVC of samples stored under *P. armeniaca* EO biocontrol for "3-4" weeks and according to Egyptian and Saudi standards, half or more of spoiled samples were listed as unacceptable for human use (58.3% of minced meat and 50.0% of skinned poultry samples). However, 66.67% of minced meat and 75.0% of skinned poultry samples stored for "1-2" weeks under *P. armeniaca* EO biocontrol were listed as acceptable as presented in Table 4.

Table 3: Fungal spoilage of examined oil treated wheat grains and tomato fruits samples under *P. armeniaca* biocontrol according to the condition of storage for 13-95 days

| Sample fungal spoilage period (days) | Examined raw food samples | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Refrigerated oil treated Samples at 4°C | | | | Unrefrigerated oil treated Samples at 25°C | | | |
| | Tomato fruits | | Wheat grains | | Tomato fruits | | Wheat grains | |
| | No. | % | No. | % | No. | % | No. | % |
| 13 | 0 | 0 | 0 | 0 | 4 | 33.33 | 0 | 0 |
| 15 | 0 | 0 | 0 | 0 | 8 | 66.67 | 0 | 0 |
| 20 | 2 | 16.67 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | 10 | 83.33 | 0 | 0 | 0 | 0 | 0 | 0 |
| 50 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 58.33 |
| 55 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 41.67 |
| 90 | 0 | 0 | 9 | 75 | 0 | 0 | 0 | 0 |
| 95 | 0 | 0 | 3 | 25 | 0 | 0 | 0 | 0 |
| Total | 12 | 100 | 12 | 100 | 12 | 100 | 12 | 100 |

*P. armeniaca: Prunus armeniaca*

## DISCUSSION

### EOs Antimicrobial Efficacy

The obtained antimicrobial properties data of 23 bioactive EOs summarized in Table 1 was in compatible with results estimated in various publications, they documented the antimicrobial efficacies of essential plant oils and extracts as same as those used in present study [1,2].

Results indicated that food-borne Gram-negative bacteria were more resistant than Gram-positive bacteria to the antibacterial properties of EOs as also deduced by Shelef [14]. It may be

attributed to the outer membrane surrounding the cell wall of Gram-negative bacteria may restrict diffusion of hydrophobic compounds through its lipopolysaccharide covering [2]. EOs from oregano, thyme, orange, anise, garlic, mustard, cinnamon, cumin and other spices have been proven in many publications to present antimicrobial activities against bacteria and/or fungi [1,2,15].

Investigated spices EOs exhibited antifungal efficacies generally more than antibacterial. This finding was also shown by Shelef who demonstrated the antimicrobial properties of spices and their EOs in culture media and found that fungi were more sensitive to spices than bacteria, and their growth and mycotoxin production are controllable in foods containing cinnamon at acceptable levels [14].

## MIC of Extracted *P. armeniaca* EO

There are many different variable methods are used for MIC determination. In present study, MIC evaluation was based on macroscopic examination of microbial growth. While Gutierrez *et al*. tested another different method, they determined MIC using the agar dilution method and/or the absorbance based microplate assay [2].

Data obtained in the dose response study was in parallel with Oussalah *et al*. who evaluated the inhibitory effect of *Corydothymus capitatus* EO on a *Pseudomonas putida* associated with meat spoilage and found that was the most active showing a MIC of 0.025% [16].

Resulted data proved that the inhibitory effects of EO increased with increasing EO concentration. This was also deduced in Celikel and Kavas study [15].

Oroojalian *et al*. studied the antibacterial effect of some EOs and recorded that the tested Gram-negative bacteria (*E. coli* 0157:H7) were the most resistant to inhibitory effect of MIC; while the most MIC efficient antibacterial activity was against *B. cereus* followed by *S. aureus* and *L. monocytogenes* [1]. While in present investigation, the least MIC was detected with *S. aureus* preceded by Gram-negative and other positive bacteria. Except with *B. sterothermophilus*, the highest MIC was effective. Present study results were also in agreement with Ouassalah *et al*. who found that *S. aureus* was the most sensitive bacteria to 26 EOs at concentrations ≤40% vol/vol [17].

Filamentous fungi exhibited high resistant against low MIC which decreased with increasing MIC. Wang *et al*. deduced a high MIC value of cinnamaldehyde against filamentous wood decay fungi (*Coriolus versicolor* and *Laetiporus sulphureus*), it was 50 and 75 ppm EO [18].

## Food Spoilage Biocontrol Effectiveness of *P. armeniaca* EO

Celikel and Kavas assessed the efficiency of five plant EOs (thyme, myrtle, laurel, sage and orange) as natural food preservatives against *E. coli*, *Listeria monocytogenes*, *S. aureus* and *Candida albicans* [15]. Their findings were in consistence with the results of this study. They found that thyme EO has a high antimicrobial efficacy against Gram-positive and Gram-negative bacteria and fungi. Also they found that EOs could be used as natural food preservatives.

Burt suggested that the low fat content of vegetables may contribute to the success of EOs applications [19]. This hypothesis is in parallel with the findings of the present study which showed that *P. armeniaca* EO has a high food preservative efficacy on all tested low fat food samples.

In nature, fungal spoilage of raw foods, especially grains, vegetables and fruits generally takes long time. So tomato and wheat samples were artificially inoculated with fungi to accelerate spoilage time. Also, beginning with high count of spoiled fungi proved the high bioactivity of *P. armeniaca* in controlling fungal food spoilage.

A study recorded the EOs antimicrobial efficacy, alone or in combination with other preservation methods, against food-borne pathogens and spoilage when applied to meat [20]. This finding confirmed the results of the current study.

Similar results documented by Belletti *et al*. who evaluated the effects of pure citral and citron EOs on microbial spoilage, growth and survival of pathogenic microorganisms during fruit-

**Table 4: Percentages of acceptable and unacceptable raw food samples under *P. armeniaca* biocontrol according to the storage time (weeks)**

| Storage time (weeks) ($n$=24) | Examined raw food samples | | | | | | | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Minced meat | | | | Skinned poultry | | | | No. | % |
| | Acceptable samples | | Unacceptable samples | | Acceptable samples | | Unacceptable samples | | | |
| | No. | % | No. | % | No. | % | No. | % | | |
| 1-2 | 8 | 66.67 | 4 | 33.33 | 9 | 75 | 3 | 25 | 24 | 100 |
| 3-4 | 5 | 41.67 | 7 | 58.33 | 6 | 50 | 6 | 50 | 24 | 100 |
| Egyptian Organization for Standardization and Production Quality (1994, 2002) | TVC<$10^6$ CFU/g | | | | TVC<$10^4$ CFUg | | | | | |
| Saudi Arabia Standards No. 1556, 1998 | TVC<$10^6$ CFU/g<br>*S. aureus*<$10^2$ CFU/g<br>*E. coli* counts: Free | | | | TVC<$10^6$ CFU/g<br>*S. aureus*<$10^2$ CFU/g<br>*E. coli* counts: Free | | | | | |

TVC: Total bacterial viable count, *S. aureus: Staphylococcus aureus, E. coli: Escherichia coli, P. armeniaca: Prunus armeniaca*

based salads storage under refrigeration. They found that both pure citral and citron EOs prolonged the microbial shelf life of the fruit-based salads. Also both oils reduced the survival of Gram-negative species *Salmonella enteritidis* and *E. coli* while inhibited the Gram-positive pathogen *L. monocytogenes* [21].

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