

Effect of Olive and Fig Leaves Extracts on the Viability of Food Poisoning Pathogens in Beef Burger

Heba M. Shaheen, Hosny A. Abdelrahman; Ali M. Ahmed; Soad A. Ismail

Dept., of Food Hygiene, Faculty of Veterinary Medicine, Suez Canal University

Abstract

Meat products are among the leading vehicles for foodborne illnesses around the world and are responsible for millions of sickening each year. Olive and fig leaf extract can be considered as a plant antimicrobial with both antibacterial and antioxidant activities they has been shown to have antibacterial activities against foodborne pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella spp.* In addition, they has been shown to enhance the quality and shelf-life of meat The average value of initial microbial load of Total aerobic count in examined beef burger samples (control group) was $9.9 \times 10^4 \pm 2 \times 10^4$ (cfu/g). where it significantly ($p < 0.05$) decreased to $4.6 \times 10^3 \pm 3 \times 10^2$, $2.8 \times 10^2 \pm 9 \times 10$ (cfu/g) by increasing the OLE concentration to (3% and 4%) and $1.3 \times 10^3 \pm 4 \times 10^2$, $2.4 \times 10^2 \pm 7 \times 10$ (cfu/g) by increasing the fig leaves extract to (4% and 5%) respectively. The average value of initial *Bacillus cereus* count of beef burger samples (control group) was $5.4 \times 10^3 \pm 1 \times 10^3$ (cfu/g). The *Bacillus cereus* count in treated samples were significantly ($p < 0.05$) decreased to $2.1 \times 10^2 \pm 1 \times 10^2$, $1.2 \times 10 \pm < 10$ (cfu/g) by increasing the OLE concentration to (3% and 4%) and $0.3 \times 10 \pm < 10$, $0.2 \times 10 \pm < 10$ (cfu/g) by increasing the fig leaves extract to (4% and 5%) respectively. The average value of initial *S. aureus* count of beef burger samples (control group) was $4 \times 10^4 \pm 2 \times 10^3$ (cfu/g). The *S. aureus* count in treated samples with OLE was significantly ($p < 0.05$) decreased to $2.3 \times 10^3 \pm 7 \times 10^2$, $8 \times 10^2 \pm 1.7 \times 10^2$ (cfu/g) by increasing the OLE concentration to (3% and 4%) respectively, while its count was not significantly ($p > 0.05$) affected in treated samples with different concentration of fig leaves extract ($1.7 \times 10^4 \pm 3 \times 10^3$, $6.7 \times 10^3 \pm 6.7 \times 10^3$ (cfu/g)) for 4% and 5% concentrations respectively. the initial load of *E. coli* in untreated samples (control group) was 80%. The incidence of *E. coli* in treated samples with OLE was significantly decreased to 15% and 5% by increasing the OLE concentration to (3% and 4%) respectively. while it was not affected by different concentrations of fig leaves extract where its incidence was 80% and 70% in treated samples with 4% and 5% fig leaves extract respectively

1- Introduction

One of the means in which foodborne pathogens can be controlled is through the use of food preservatives, which can be classified as synthetic chemical and natural antimicrobial compounds. Compared to synthetic food preservatives, plant antimicrobials attract more attention since they are generally recognized as safe, and may benefit to human health (Seowet *et al.*, 2014). Moreover, plant antimicrobials may also add flavor foods. Most of consumers are aware about the side effects and hazards of this chemical additives which added to meat products (Bin *et al.*, 2007). Therefore the food manufacturer tried to use natural substances of plant origin that have antimicrobial and seasonings properties instead of using chemical preservatives.

Olive and fig trees are believed to be one of the oldest cultivated plants in Egypt which is considered one of the major producers of olive and fig worldwide. Olive trees can be found in several areas in Egypt North Sinai, the Alexandria-Cairo road, Siwa and Fayoum while more than 50% of the total fig area is located along the north western coast of Alexandria as well as Sinai Governorates. Plant parts and extracts of these trees have traditionally been used in many

application especially their leaves which are believed to have a preservative and medicinal properties.

The antimicrobial activities of olive leaves and fig leaves are due to their contents of phenolic components which have more inhibitory effect against Gram-positive and Gram-negative bacteria (Liu *et al.*, 2017). Olive and fig leaf extract can be considered a plant antimicrobial with both antimicrobial and antioxidant activities (Soltana *et al.*, 2016) they has been shown to have antimicrobial activities against foodborne pathogens such as *Staphylococcus aureus*, *E. coli*, *Salmonella spp.* In addition, they has been shown to enhance the quality and shelf-life of meat products (Maghsoudlou *et al.*, 2017). Therefore this study was carried out for assessment of the antimicrobial activities of olive and fig leaves extract against the isolated bacterial group in beef burger with different concentrations of plant extract and its effect on sensory and bacteriological quality.

2- Materials and Methods

2.1 Collection of plant materials

Fresh green olive (*Olea europaea L.*) and fig (*Ficus carica*) leaves were collected from north Sinai and the farm of Faculty of Agriculture, Suez Canal University, Egypt respectively.

2.2. Preparation of the plant extract

The leaves were cleaned from extraneous matter and properly washed then dried in hot air-oven for 24 hrs at 40°C. The dried leaves were ground in a blender to form powder. Thereafter, 100g of the powder of each plant were macerated in 1000 ml absolute ethanol and allowed to extract for 48 h (Dub and Dugani, 2013). The resultant (dark green-brown mixture) was filtered and the filtrate was concentrated in a rotary evaporator under reduced pressure. The extraction yield % of the alcoholic extract of olive and fig leaves were 14% and 11% respectively. The dried extract residue was reconstituted in sterile propylene glycol to give final concentration of 3%, 4% for olive leaves extract and 4%, 5% for fig leaves extract.

2.3. Determination of antimicrobial activity of the prepared extract

The antibacterial activity of the extract was measured by disc diffusion test using Mueller–Hinton agar previously inoculated with 1 mL of 18 h old of bacterial suspension (10^6 CFU/mL) (Changwee *et al.*, 2008). Sterilized paper discs (6 mm) were impregnated with 20 μ L of different concentrations of extracts and placed onto the Mueller–Hinton agar plates. The plates were incubated at 4°C for 2hrs to allow diffusion of the active compounds in the medium (Tagg and Mcgiven, 1971). Negative controls were prepared using the same solvent employed to dissolve the plant extract. Gentamicin discs (10 μ g, Oxoid, UK) were used as control and positive controls. Incubation of plates was performed at 37°C for 24hrs. Inhibition zones in mm (without disc paper diameter) around discs were measured. The antibacterial activity was expressed as the diameter of inhibition zones produced by the extract against test microorganisms. The experiment was repeated in triplicate and the mean of diameter of the inhibition zones was calculated.

2.4. Beef patties preparation / experimental design:

Beef patties preparation was carried out in agreement with Egyptian standard specification for burger (ES 1688/2005) as where it was formulated with 65% minced meat, 20% fat, 5% soybean, 0.3% black pepper, 1.8% salt and 10% cool iced water to achieve the good manufacturing practices for beef burger.

Twenty kilograms of freshly beef chuck 24 hours postmortem was purchased from local butcher shop at Ismailia market-Egypt and directly transported to the laboratory in an ice box to be minced in electrical mincer (5mm). Fresh beef fat was immediately purchased from a local slaughterhouse after slaughtering and carcass preparation; washed and kept frozen at -18⁰C for the next day. Before processing of the burger, the beef fat was immediately minced using electrical mincer at 3mm diameter.

Frozen minced beef was transferred to a paddle mixer, where common salt, black pepper and cold water were added and thoroughly mixed for five minutes and divided into five portions. First portion was used as control, while the other portions were mixed with the prepared concentrations of olive and fig leaves extract (3%, 4% for OLE and 4%, 5% for fig leaves extract). The obtained pastes were formed into 50g beef burger using cardboard meat box, packed in foam plates and stored at refrigerator shelf at 4°C. 20 samples for each treatment were examined for sensory and microbiological quality evaluation.

2.5. Bacteriological evaluation:

Samples homogenate and serial decimal dilutions were prepared following the recommendation of **APHA (2001)**. The serial dilutions of each sample were examined for evaluation the effect of the added extracts on:

- 1- Determination of Total Aerobic Count. **ISO(2003b)**
- 2- Determination of *B. cereus* count. **ISO(2004a)**
- 3- Determination of *S. aureus* count. **ISO(1999a)**
- 4- Detection of *E. coli*. **ICMSF(1996)**

2.6-Statistical analysis

Data analysis was performed by using SPSS statistical software program (**SPSS for Windows version 16, Spss Inc., USA**). Data were expressed as mean \pm standard error (SE). One-way analysis of variance (ANOVA) with Duncan post-hoc multiple comparisons test. Any significant differences ($P < 0.05$) were analyzed by the multiple comparisons procedure of LSD (least significant difference), using a level of significance of $\alpha = 0.05$.

3- Results and Discussion

The antibacterial effect of different concentrate on of olive and fig leaves extracts was evaluated against the isolated bacterial group in beef burger in addition to evaluating its effect on sensory and bacteriological quality.

Olive and fig leaves extract can be considered a plant antimicrobial with both antimicrobial and antioxidant activities. They has been shown to have antimicrobial activities against foodborne pathogens such as *Staphylococcus aureus*, *E. coli*, *Salmonella* spp. In addition, they have been shown to enhance the quality and shelf-life of meat products.

3.1. Total aerobic count:

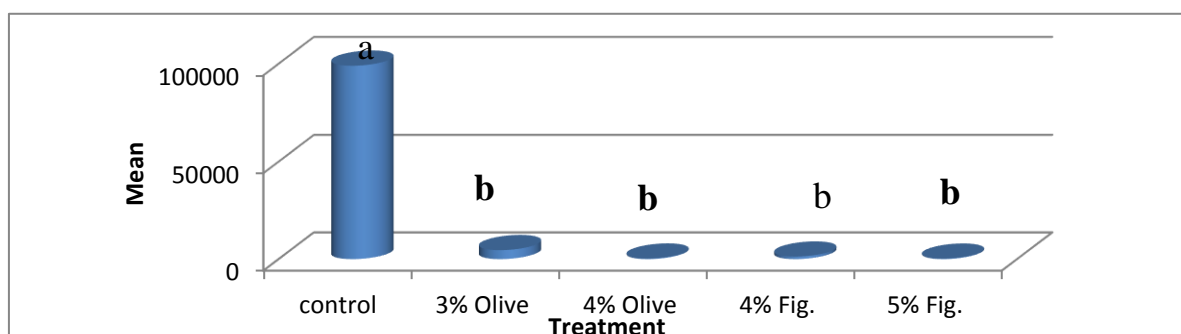


Figure (1): Statistical analytical results for the effect of olive and fig leaves extract on Total Aerobic Count (CFU/g) in beef burger (n=20). Columns have different letters are significantly different ($p < 0.05$).

Results obtained in fig (1) show the effect of olive and fig leaves extract on total aerobic count (cfu/g) in beef burger where its count was ranged from 4×10^4 to 2×10^5 , 3×10 to 3×10^4 , 5×10 to 1×10^3 , 1.3×10^2 to 5×10^3 and 6×10 to 8×10^2 (cfu/g) for control group, 3% olive, 4% olive, 4% fig and 5% fig leaves extract treatments respectively. The average value of initial microbial load of beef burger samples (control group) was $9.9 \times 10^4 \pm 2 \times 10^4$ (cfu/g). The TACs in treated samples were significantly ($p < 0.05$) decreased to $4.6 \times 10^3 \pm 3 \times 10^2$, $2.8 \times 10^2 \pm 9 \times 10$ (cfu/g) by increasing the OLE concentration to (3% and 4%) and $1.3 \times 10^3 \pm 4 \times 10^2$, $2.4 \times 10^2 \pm 7 \times 10$ (cfu/g) by increasing the fig leaves extract to (4% and 5%) respectively.

Moreover there was a non-significant difference in TACs for treated groups ($p > 0.05$) while results obtained in figure (2) show the reduction percent of total aerobic count (cfu/g) in treated beef burger with olive and fig leaves extract where the highest reduction percent (99.7%) was achieved by 4% OLE and 5% fig leaves extract followed by 4% fig leaves (98.8%) extract then 3% OLE (95%).

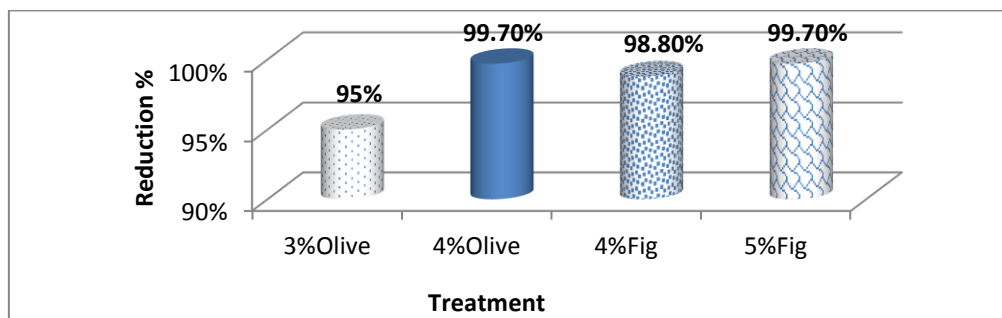


Fig. (2): Reduction percent of Total Aerobic Count (CFU/g) in treated beef burger with olive and fig leaves extract.

Nearly similar results was obtained by Aytulet *et al.* (2008), Aytul (2010), Aliabadi, *et al.* (2012) Ahmed *et al.* (2014), Kirazet *et al.* (2016), Moawadet *et al.* (2017) and Nirwanaet *et al.* (2018)

3.2. *Bacillus cereus* count:

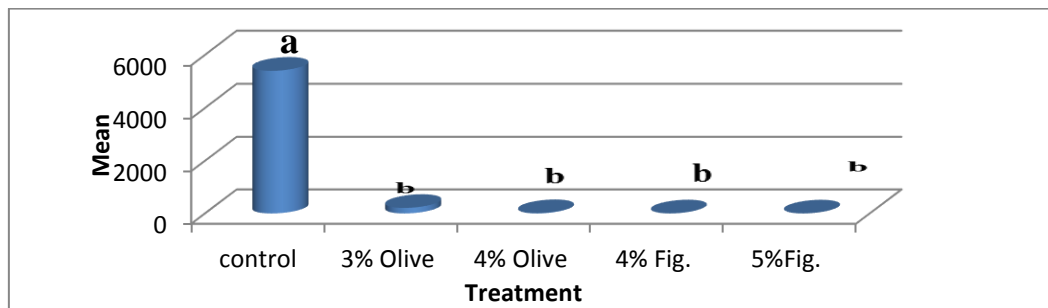


Figure (3): Statistical analytical results for the effect of olive and Fig leaves extract on *Bacillus cereus* (CFU/g) in beef burger (n=20). Columns have different letters are significantly different ($p < 0.05$).

Results obtained in fig (3) show the effect of olive and fig leaves extract on total *Bacillus cereus* count (cfu/g) in beef burger where its count was ranged from < 10 to 2×10^4 , < 10 to 1×10^3 ,

<10 to 1×10^2 , <10 to 2×10 and <10 to 1×10 (cfu/g) for control group, 3% olive, 4% olive, 4% fig and 5% fig leaves extract treatments respectively. The average value of initial *Bacillus cereus* count of beef burger samples (control group) was $5.4 \times 10^3 \pm 1 \times 10^3$ (cfu/g). The *Bacillus cereus* count in treated samples were significantly ($p < 0.05$) decreased to $2.1 \times 10^2 \pm 1 \times 10^2$, $1.2 \times 10 \pm <10$ (cfu/g) by increasing the OLE concentration to (3% and 4%) and $0.3 \times 10 \pm <10$, $0.2 \times 10 \pm <10$ (cfu/g) by increasing the fig leaves extract to (4% and 5%) respectively.

Moreover there was a non-significant difference in *Bacillus cereus* count for treated groups ($p > 0.05$) while results obtained in figure (4) show the reduction percent of *B. cereus* count (cfu/g) in treated beef burger with olive and fig leaves extract where the highest reduction percent (99.96%) was achieved by 5% fig leaves extract followed by 4% fig leaves extract (99.94%) then 4% OLE (99.70%) while the lowest reduction percent (96%) was obtained by 3% OLE.

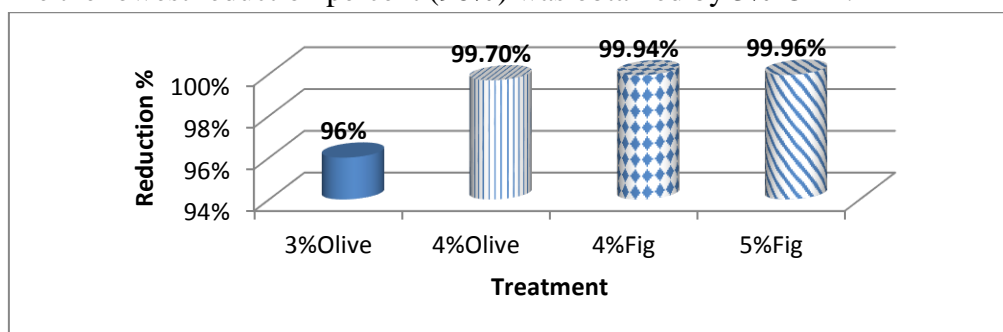


Fig. (4): Reduction percent of *Bacillus cereus* (CFU/g) in treated beef burger with olive and fig leaves extract

Nearly similar results was obtained by Owen *et al.*, (2003), Pereira *et al.*, (2007), Keskinet *al.*, (2012), Gökmenet *al.*, (2014) who found that OLE displayed the best activity against *B. cereus* while lower results was obtained by Ahmed *et al.* (2013) who found that methanolic extract of *F. carica* leaves exhibited the minimum antibacterial effect against *B. cereus* compared with other tested bacteria.

3.3. *Staphylococcus aureus* count:

Results obtained in fig. (5) showed the effect of olive and fig. leaves extract on total *S. aureus* count (cfu/g) in beef burger where its count was ranged from 2×10^3 to 17×10^4 , 1×10^2 to 7×10^3 , 7×10 to 2×10^3 , 5×10^3 to 3×10^4 and 1.3×10^3 to 1.3×10^4 (cfu/g) for control group, 3% olive, 4% olive, 4% fig and 5% fig leaves extract treatments respectively. The average value of initial *S. aureus* count of beef burger samples (control group) was $4 \times 10^4 \pm 2 \times 10^3$ (cfu/g).

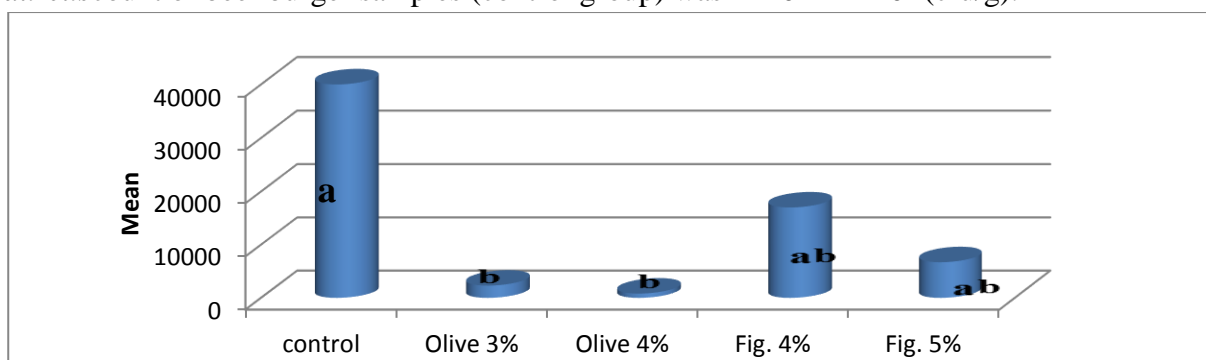


Fig.(5): Statistical analytical results for the effect of olive and fig leaves extract on *Staphylococcus aureus* (CFU/g) in beef burger (n=20). Columns have different letters are significantly different ($p < 0.05$).

The *S. aureus* count in treated samples with OLE was significantly ($p < 0.05$) decreased to $2.3 \times 10^3 \pm 7 \times 10^2$, $8 \times 10^2 \pm 1.7 \times 10^2$ (cfu/g) by increasing the OLE concentration to (3% and 4%) respectively, while its count was not significantly ($p > 0.05$) affected in treated samples with different concentration of fig leaves extract ($1.7 \times 10^4 \pm 3 \times 10^3$, $6.7 \times 10^3 \pm 6.7 \times 10^3$ (cfu/g)) for 4% and 5% concentrations respectively.

Moreover there was a non-significant difference in *S. aureus* count for treated groups ($p > 0.05$) while results obtained in figure (6) show the reduction percent of *S. aureus* count (cfu/g) in treated beef burger with olive and fig leaves extract where the highest reduction percent (98%) was achieved by 4% OLE followed by 3% OLE (94%) then 5% fig leaves extract (83%) while the lowest reduction percent (57.5%) was obtained by 4% fig leaves extract.

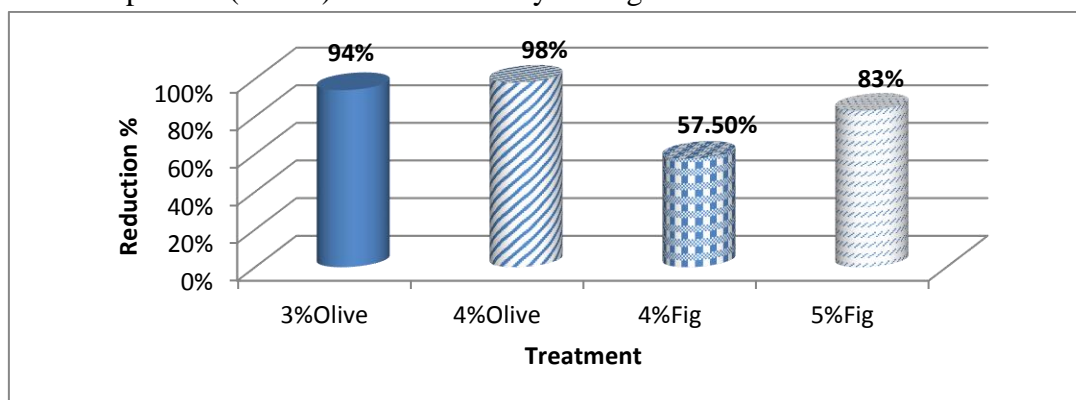


Fig. (6): Reduction percent of *Staphylococcus aureus* (CFU/g) in treated beef burger with olive and fig leaves extract

The obtained results was nearly similar to those obtained by **Owen *et al.*, (2003)**, **Pereira *et al.*, (2007)** and **Gökmen *et al.*, (2014)** who found that OLE has a strong antibacterial effect against *S. aureus* while it disagree with **Jeon *et al.*, (2009)**, **Al Askari *et al.*, (2013)** and **Rashid and Mahdi (2014)** who found that fig leaves extract has an inhibitory effect against *s. aureus*

3.4. Detection of *E. coli*:

Results in figure (7) show the effect of olive and fig leaves extract on *E. coli* (CFU/g) in beef burger, where the initial load of *E. coli* in untreated samples (control group) was 80%.

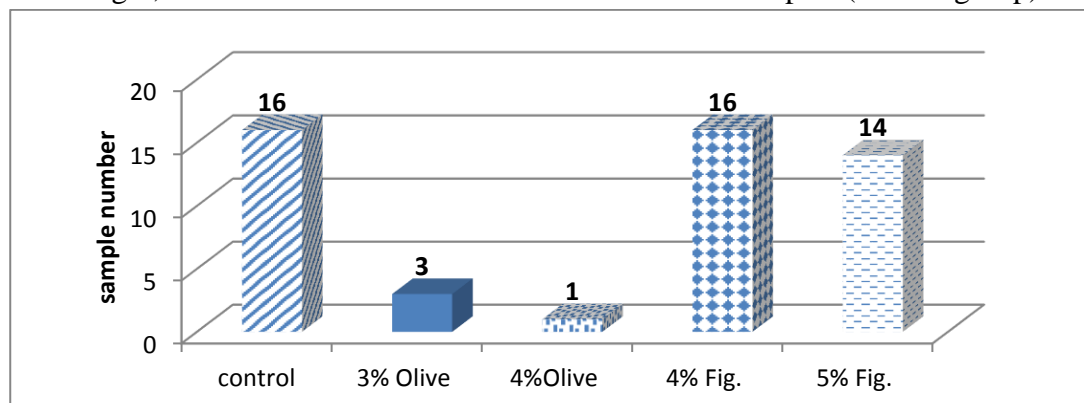


Fig. (7): Positive samples for presence of *E. coli* in treated beef burger samples (n=20)

The incidence of *E. coli* in treated samples with OLE was significantly decreased to 15% and 5% by increasing the OLE concentration to (3% and 4%) respectively. These results agreed with **Markinet *al.*, (2003)** **Gökmenet *al.*, (2014)**, **Liu *et al.*, (2017)** while it was not affected by different concentrations of fig leaves extract where its incidence was 80% and 70% in treated samples with 4% and 5% fig leaves extract respectively. These results indicate that the activity of fig leaves extracts was more effective against Gram-positive than Gram-negative bacteria; this fact is in agreement with **Alzoreky and Nakahara (2003)**, **Nihalet *al.*, (2007)**, **Al Askariet *al.*, (2013)**, **Rashid and Mahdi (2014)** and **Mahmoudiet *al.*, (2016)**

The higher resistance of Gram-negative bacteria against plant extracts is credited to the presence of outer membrane lipopolysaccharides. Also these observations are likely to be the consequences of the differences in cell wall structure between Gram-positive and Gram-negative bacteria. Thus the Gram-negative outer membrane can act as a barrier against many environmental substances, including antibiotics.

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الملخص العربي

تأثير مستخلصات أوراق الزيتون والتين على قابلية البكتيريا المسببة للتسمم الغذائي في بيرجر اللحم

هبة محمد شاهين وحسني عبداللطيف عبدالرحمن وعلي معوض احمد وسعاد احمد اسماعيل
قسم الرقابة الصحية علي الاغذية - كلية الطب البيطري - جامعة قناة السويس

تعد منتجات اللحوم من اهم المصادر المسببة للتسمم الغذائي حول العالم. يعتبر مستخلص اوراق التين والزيتون من المواد الطبيعية التي لها تأثير مضاد للبكتيريا ومضاد للاكسدة فقد اثبتت النتائج ان لهم تأثير مضاد للعديد من بكتيريا التسمم الغذائي مثل بكتيريا العنقود الذهبي، الاشيرشيا كولاي و الباسيلس سيرس. بينما اوضحت نتائج التقييم البكتريولوجي لعينات البيرجر التي تم فحصها (20 عينة) ان متوسط الحمل الميكروبي الاولي للمجموعة الضابطة في حالة الجراثيم الهوائية كان $10 \times 9.9 \pm 2 \times 10^4$ جرثومة لكل جرام والذي انخفض انخفاض معنوي الى $10 \times 4.6 \pm 3 \times 10^3$ و $10 \times 2.8 \pm 2 \times 10^3$ جرثومة لكل جرام في عينات البيرجر المعالجه بمستخلص اوراق الزيتون مع زياده التركيز الى 3% و 4% بينما انخفض الى $10 \times 1.3 \pm 3 \times 10^2$ و $10 \times 2.4 \pm 2 \times 10^2$ جرثومة لكل جرام في عينات البيرجر المعالجه بمستخلص اوراق التين مع زياده التركيز الى 4% و 5% على التوالي و لم يكن هناك فروق معنوية بين المجاميع المعالجه. وفي حالة الباسيلس سيرس اوضحت النتائج ان متوسط الحمل الميكروبي

الاولى للمجموعه الضابطه كان $10 \times 5.4 \pm 1 \times 10^3$ جرثومه لكل جرام والذي انخفض انخفاض معنوى الى $10 \times 2.1 \pm 5 \times 10$ و $10 \times 1.2 \pm 10$ جرثومه لكل جرام فى عينات البيرجر المعالجه بمستخلص اوراق الزيتون مع زياده التركيز الى 3% و 4% بينما انخفض الى $10 \times 0.3 \pm 10$ و $10 \times 0.2 \pm 10$ جرثومه لكل جرام فى عينات البيرجر المعالجه بمستخلص اوراق التين مع زياده التركيز الى 4% و 5% على التوالى و لم يكن هناك فروق معنويه بين المجاميع المعالجه. وفى حاله بكتريا العنقود الذهبى اوضحت النتائج ان متوسط الحمل الميكروبي الاولى للمجموعه الضابطه كان $10 \times 4 \pm 2 \times 10^3$ جرثومه لكل جرام والذي انخفض انخفاض معنوى الى $10 \times 2.3 \pm 7 \times 10^2$ و $10 \times 8 \pm 1.7 \times 10^2$ جرثومه لكل جرام فى عينات البيرجر المعالجه بمستخلص اوراق الزيتون مع زياده التركيز الى 3% و 4% بينما لم يكن هناك انخفاض معنوى فى متوسط الحمل الميكروبي لبكتريا العنقود الذهبى فى العينات المعالجه بمستخلص اوراق التين فكانت $10 \times 1.7 \pm 4 \times 10^3$ و $10 \times 6.7 \pm 3 \times 10^3$ جرثومه لكل جرام مع زياده التركيز الى 4% و 5% على التوالى. لم يكن هناك فروق معنويه بين المجاميع المعالجه كما اوضحت النتائج ان نسبه تواجد الاشيريشيا كولاي فى المجموعه الضابطه كان 80% والذي انخفض انخفاض معنويا الى 15% و 5% فى العينات المعالجه بمستخلص اوراق الزيتون مع زياده التركيز الى 3% و 4% على التوالى بينما لم تتاثر بالتركيزات المختلفه لمستخلص اوراق التين فكانت نسبه تواجدها 80% و 70% فى العينات المعالجه بتركيز 4% و 5% على التوالى.