Evaluation of the Bioactivities of Plant Extractions from Lavandula stoechas L. and Phlomis syriaca Boiss on Some Pathogenic Microorganisms Isolated from Local Fresh Red Meat

تقييم الفعالية البيولوجية للمستخلصات النباتية لنباتي (الضِّرْمُ المكور) Lavandula stoechas و(الأذينة السورية) Phlomis syriaca على بعض الكائنات الدقيقة الممرضة المعزولة من اللحوم الحمراء المحلية

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ABSTRACT

Plant extracts have become increasingly important additives in the food industry because of their anti-microbial capacity in processed meat products due to their natural origin. They are an excellent candidate to replace synthetic molecules, which are generally considered to have toxic and carcinogenic effects. The effective extraction of these antioxidant molecules from their natural sources and identifying their activity in commercial products is a major challenge for researchers and contributors to food processing. Pathogenic bacteria were isolated from red meat and study the effect of water extracts of L. stoechas and Phlomis syriaca plants in different concentrations on pathogens. The application of plant extracts to improve shelf life and nutritional and health characteristics of red meat products. The activities of the water extracts of the L. stoechas wild plant on the bacteria isolated from meat. The water extract of the L.stoechas plant, with a concentration of 1%, showed positive results on all bacteria ranging between 11 and 15 mm. The sensitivity to the Klebsiella extract seemed higher than other bacterial strains, where the inhibition halo was 15 mm in diameter. In contrast, the Listeria was resistant to the Lavandula water extract with concentrations of 0.5 and 0.25%. The 1% concentration of the water extract of the Phlomis syriaca also showed positive results on all bacteria ranging from 10 to 15 mm. The sensitivity of the Pseudomonas aeruginosa, shigella and salmonella appeared high in terms of the extract compared to other bacterial strains. The halo of inhibition was 15 mm with a concentration of 1% of the water extract. For the Phlomis syriaca, Listeria, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus were resistant to the water extract of the Phlomis syriaca at a concentration of 0.25%.

Keywords*:* Wild plants, Lavandula stoechas, Phlomis syriaca, red meat, water extracts, pathogenic microbes.

الملخص

تستأثر المستخلصات النباتية بأهمية متزايدة كمضافات مهمة في صناعة المواد الغذائية؛ بسبب مقدرتها المضادة للجراثيم في منتجات اللحوم الجاهزة للأكل، فهي مرشحة ممتازة لتحل محل الجزيئات المُصّنعة ذات التأثير السام والمسرطن، وكان الاستخراج الفعال لهذه الجزيئات المضادة للأكسدة من مصادرها الطبيعية إلى جانب تحديد نشاطها في المنتجات التجارية يمثّل تحدِّياً كبيراً للباحثين والمساهمين في التصنيع الغذائي. تم عزل الأحياء الدقيقة الممرضة من اللحوم ودراسة تأثير المستخلصات المائية لنباتي الضرم المكور والأذينة السورية بتراكيز مختلفة على الأحياء الدقيقة الممرضة. نشاطات المستخلصات المائية لنبات (الضرم المكور L.stoechas) البرية على الجراثيم المعزولة من اللحوم الحمراء ، حيث أبدى المستخلص المائي لنبات (الضرم المكور) بتركيز (1%) نتائج إيجابية على الجراثيم جميعها تراوحت بين(11 و 15 مم) وبدت حساسية(الكلبسيلة Klebsiella) عالية للمستخلص مقارنة بالسلالات الجرثومية الأخرى حيث بلغ قطر هالة التثبيط (15 مم)، في حين أبدت الليسترية Listeria مقاومة للمستخلص المائي للضرم بالتركيزين(0.5 و0.25%). كما أظهر التركيز (1%) للمستخلص المائي (للأذينة) السورية نتائج إيجابية على الجراثيم تراوحت بين (10 و15 مم)، وبدت حساسية الزائفة الزنجارية والشيغلة والسلمونيلة عالية للمستخلص مقارنة بالسلالات الجرثومية الأخرى، وبلغ قطر هالة التثبيط (15 مم) بتركيز (1%) للمستخلص المائي للأذينة السورية، وأبدت الليسترية والإشريكية القولونية والزائفة الزنجارية والعنقوديات الذهبية مقاومة للمستخلص المائي (للأذينة) السورية بتركيز (0.25%). تطبيق المستخلصات النباتية لتحسين مدة الصلاحية، والخصائص الغذائية والصحية لمنتجات اللحوم الحمراء.

الكلمات المفتاحية: الفصيلة الشفوية، الضرم المكور، الأذينة السورية، اللحوم الحمراء، المستخلصات المائية، الجراثيم الممرضة.

INTRODUCTION

Food poisoning is one of the most common causes of disease and death in developing countries (Doughari and Pukuma, 2007; Pirbalouti A. G. *et al.*, 2010; Sapkota R. *et al.*, 2012) due to contamination with gram-negative bacteria, such as *Salmonella typhi and Escherichia coli, Escherichia coli, Pseudomonas aeruginosa* (Solomakos N et al., 2008; Pandey A., Singh P. 2011), and Gram-positive bacteria, such as *Staphylococcus aureus and Bacillus cereus*.

However, preservative chemicals can be used to prevent bacterial growth and prevent food spoilage (Yamamura A et al., 2000). The control of food-borne pathogens requires the use of various preservation techniques in the manufacture and storage of food products. Therefore, there is an increasing need for active preservatives that can prolong product life, inhibiting non-salt microbial growth and sugar, which the consumer prefers at low levels (Zink, 1997). The negative perception of synthetic food additives and the emergence of negative side effects in health led to the development of natural alternatives and the use of extracts pate and oils antibacterial agents, which are safer and more active (Bialonska D et al., 2010, Tajkarimi M. M. et al., 2010; Hussain M et al., 2018).

On the other side, essential oils or their components have antibacterial properties (Oussalah M et al., 2007), parasites (George et al., 2009), viruses (Astani A. et al., 2011), fungi (Silva F et al., 2011; Tiemersma et al., 2011) and antifungal agents. However, the use of vegetable oils and extracts as food preservatives requires knowledge of their Minimal Inhibition Concentration (MIC).

Meat products are oxidized, especially when stored. Mincing facilitates the interaction of oxidants with unsaturated fatty acids and the formation of free, which negatively affects color, flavor, texture, and food quality (Vaithiyanathan S et al., 2011). Fortunately, the application of antioxidants prevents fat oxidation (Martinez-Tome et al., 2001), and plants are of great importance as natural additives (Lindberg, Bertelsen 2011; Zheng and Wang, 2001).

RESEARCH PROBLEM

There is a lack of local studies on wild plants, despite their spread in different Syrian environments. It hinders efforts in determining the extent of the effect of their extracts on pathogenic or non-pathogenic bacteria. It also afflicts the utilization of plant compounds as substances with biological effectiveness and as alternatives to biological antibiotics.

RESEARCH SIGNIFICANCE

Natural alternatives are important for preserving food, such as plant extracts and essential oils with their anti-microbial properties that have a negative effect on food composition and their nutritional quality. Therefore, studying the effect of Syrian lavender and atrium as meat preservatives, preventing microorganism corruption or transfer to pathogens, and prolonging their storage is of great importance.

RESEARCH AIMS

The aim of this research is to identify the anti-aqueous activity of L. stoechas plants and Phlomis syriaca on some bacteria isolated from red meat and preserve them as bioactive and alternative substances for antibiotics and synthetic chemicals.

MATERIALS AND METHODS

Plant Materials

The samples of the L. stoechas and Phlomis syriaca selected for the research were collected in the village of Qismin from the North Kabir area below Tishreen Lake, west of the riverbed at an altitude of about 100-170 m above sea level, 20 km northeast of Lattakia and north of Aleppo road. The sample was sent to the laboratory, washed with sterile distilled water, and then dried on filter paper at 25°C.

L. stoechas, in Figure 1, belongs to the oral family Oblige letter, a herb that spreads in the Mediterranean and the Black Sea areas, on sunny slopes and favors silicic soils. It does not grow in the shade and is relatively tolerant to drought. It is a small branched shrub of 30-50 cm long and has flowers. In general, the coronets are purple, flowering from February to May (ACSAD, 2012).

Figure 1. Lavandula stoechas L.

The Syrian atrial plant *Phlomis syriaca,* Figure 2, belongs to the oral family. This genus' species home is the Mediterranean basin and from Central Asia to China. The flowers are yellow or violet and bloom during the period from April to June. Various studies have indicated the medicinal value of the plant. It enhances the protection of the liver, kidneys, bones, heart, and blood vessels. It is also antibacterial, anti-allergic, and has antioxidant properties (Demirci et al., 2008).

Figure 2. Part of *Phlomis syriaca* Boiss

Preparation of Plant Extracts

Aqueous extracts

Previously dried leaves were placed in the incubator for two hours at 37°C to eliminate any moisture in the sample for easy grinding of the leaves (Blazekovic et al., 2011). The dry leaves were ground with an electric grinder, 25g of the powder was taken and soaked in a 500ml flask with 250ml of distilled water (Khayyat et al., 2018). The samples were placed in the shaker at 170 rpm at laboratory temperature (25°C), away from light for 48 hours (Mahmoudi et al., 2014). The extract was filtered using a 0.45 Watman filter paper and dried in an air incubator at a temperature not exceeding 40°C.

Isolation of pathogens from red meat

The researchers took 10 samples of lamb and 10 samples of veal, Figure 3. Ten grams of each sample were added to 100 ml of sterile peptone, then mixed for 3 minutes at high speed, from 10-1 to 10-4, after they have compiled the analysis of the Hungarian neighborhoods (i.e., vaccination).

Pathogenic microorganisms were isolated from meat samples and a selection of selective solid bacterial media was taken, such as EMB Agar for Enterobacter development, Chapman Agar for staphylococcal development, and CetramideAgar to characterize Pseudomonas aeruginosa Editing ammonia, Agar PDA Dextrose Potato medium for the development of Candida albicans, as well as nutritious agar medium and nutrient broth (APHA, 2000). Bacteria isolated from the samples could be classified after conducting the necessary biochemical tests (oxidase, catalase, indole, methyl red, citrate fermentation, VogsProscaur, H2S release) based on the Bergi evidence (Garrity G. M et al., 2005).

Figure 3. Lamb and veal samples

Furthermore, the sensitivity and resistance of isolated microbes were tested by the propagation method of tablets. The sensitivity and resistance of some antibiotics were determined by measuring halos inhibitors on Mueller Hinton Agar's medium (Barker and Kehoe E, 1995).

A group of antibiotics was used (Table 1) with a concentration in the tablets.

Table 1Used Antibiotics, mcg.

Antibiotc	Code
Ampicillin/ Cioxacillin	APX: 25/5 mcg
Lincomycin	L:2 mcg
Erythromycin	E:15 mcg
Cefaclor	CEC: 30 mcg
Pipemidic	PI:20 mcg

Preparation of bacterial inoculum

The activation of activated microbes was carried out on the Nutrient Agar NA and incubated at 37°C for 24 hours. Ten colonies of each type of bacterium were transferred, under sterile conditions, to a test tube containing 5 ml of broth nutrients. At 37°C for 4 hours, appropriate dilutions were carried out for each type of bacterium, with a total cell count of 1.5 x 8^{10} cells/ml (Anandiand Juan, 2009).

Test of anti-microbial Bioactivity of Plant Extraction

Aqueous extracts were prepared at a concentration of 0.25, 0.5, 0.75, and 1.0%. Muller-Hinton medium was prepared by dissolving 38 g of it in a liter of distilled water, with 0.5 ml of bacterial suspension, and brushes were transferred over the culture medium with a cotton swab. After 15 minutes, the extracts were distributed and placed in the fridge. The incidence of inhibition zones is evidence of bacterial growth inhibition, and their diameters are measured after finishing the lap with a precise measuring instrument or millimeter ruler. The experiment was performed with three replications (Kelmanson et al., 2000).

RESULTS AND DISCUSSION

The Effect of Dry Extracts of *Lavandula stoechas* L. and ***Phlomis syriaca***

The results shown in Table 2 showed that the *L. stoechas* plant contains 22.98% of the pure extract of aqueous extract. In comparison, the *Phlomis syriaca* plant contains 21.11% of the pure extract.

Table 2 The Yield of Dry Plant Extracts Used in the Research.

Plant	the sample	Repeater% 1	2	3	Average and deviation
Lavandula stoechas	25	23.56	22.4	22.98	22.98 ±0.58
Phlomis syriaca	25	20.5	21.73	21.11	21.11± 0.61

Bacterial species isolated from meat

Table 3 shows the six isolated bacterial species when testing red meat microbiologically and one type of yeast *Candida albicans.* See the *Pseudomonas aeruginosa, E.coli* in Figure 5, the *Klebsiella* in Figure 4 and the *Shigella* (Table 3).

Table 3 Microorganisms Isolated from Red Meat from Different Areas in Homs

Microbe strain	Rate of positive samples in meat% Sheep meat	veal
E. coli	85.71	66.66
Pseudomonas aeruginosa	57.14	33.33
Klebsiella sp.	42.85	16.66
Shigella sp.	28.57	50.00
Salmonella sp.	42.85	33.33
Staphylococcus aureus	14.28	66.66
Candida albicans	28.57	16.66

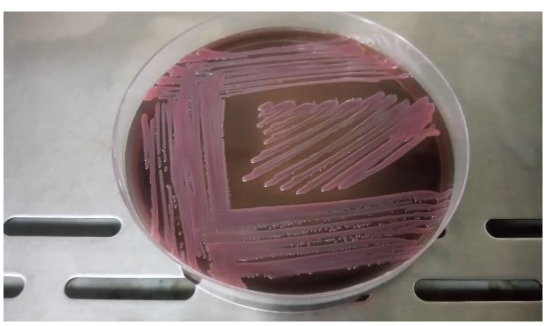

Figure 4. *Klebsiella* on EMB

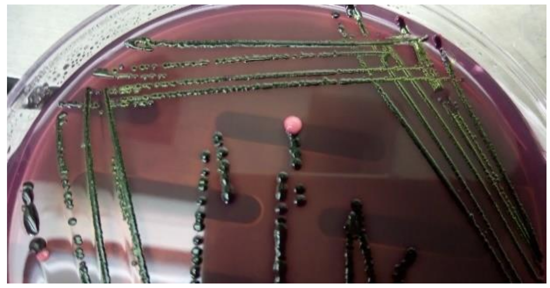

Figure 5. *E. coli* on EMB

Biological activity of L.stoechas plant extracts on pathogenic microorganisms.

Table 4 shows the results of the study of the biological activity of water extract of the L. stoechas against germ

Table 4 Diameter of Halo Inhibition of Aqueous Extract of the Wild Plant L. stoechas for Tested Germs and Candida albicans, per mm

Microbe strain	Average halo inhibition diameter,% 0.25	0.5	0.75	1.0
E. coli	0.0 ± 7	9 ± 0.0	12 ± 1.0	14 ± 0.57
Pseudomonas aeruginosa	6 ± 0.0	10 ± 1.0	11 ± 0.57	13 ± 1.0
Klebsiella sp.	-	8 ± 1.52	12 ± 0.0	15 ± 1.52
Shigella sp.	-	09 ± 1.0	11 ± 0.57	12 ± 2.08
Salmonella sp.	6 ± 1.0	8 ± 0.57	12 ± 0.57	14 ± 1.52
Staphylococcus aureus	7 ± 1.0	9 ± 0.0	12 ± 1.0	13 ± 0.0
Listeria sp.	-	-	9 ± 1.0	11 ± 0.0
Candida albicans	7 ± 0.57	10 ± 1.0	11 ± 0.0	14 ± 0.0

Moreover, the concentration of 1% showed positive results on all bacteria ranging from 11 to 15 mm (Figure 6). The klebsiella sensitivity of the extract was high compared to other bacterial strains. The inhibition of 9 mm in concentration was 0.75%, which is consistent with the result of the researchers (Tabatabaei et al., 2014). The maximum value (14 mm) was 1.0%.

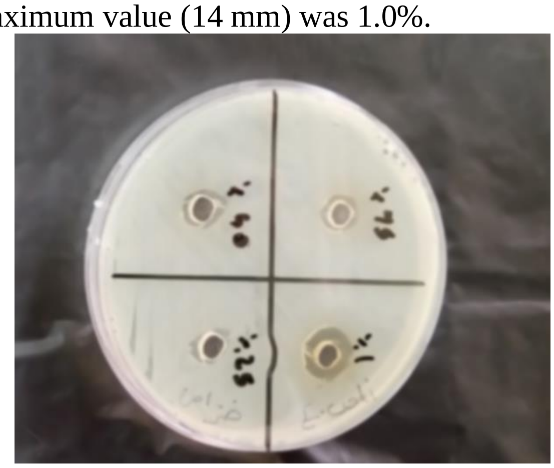

Figure 6. The efficacy of L. stoechas water extract on E.coli

Table 5 shows the results of the study of the impact of the biological activity of the aqueous extract of the *Boiss* against bacteria.

Table 5 Diameter of Halo Inhibition of Aqueous Extract of Syrian Atrial Plant Phlomis syriaca for Tested Bacteria and Candida albicans, per mm

Microbe strain	Average halo inhibition diameter,%			
	0.25	**0.5**	**0.75**	**1**
E. coli	-	7± 0.0	9± 1.0	10± 0.0
Pseudomonas aeruginosa	-	8± 0,57	10± 0.0	15± 1.0
Klebsiella sp.	6± 1.0	7± 1.52	9± 0.0	10± 1.52
Shigella sp.	-	9± 1.0	11 ± 0.57	15± 2.08
Salmonella sp.	06 ± 1.0	8± 0.0	10± 0.57	15± 1.52
Staphylococcus aureus	-	7± 0.0	10± 1.0	12± 0.0
Listeria sp.	-	6± 1.0	8± 1.0	10± 0.0
Candida albicans	6±1.0	9± 1.0	11 ± 0.0	14 ± 0.0

The concentration of 1% showed positive results on all germs ranging from 10 to 15 mm. The sensitivity of Pseudomonas aeruginosa in Figure 7. The Shigella and Salmonella appeared to be high for the extract compared to other bacterial strains. Gold resistance to aqueous extract of the Boiss was at a concentration of 0.25%.

Table 6 shows the results of the sensitivity of the isolated pathogenic bacteria to the tested biological antibiotics. All the bacteria under test were resistant to Ampicillin, Cefaclor, Lincomycin, and Erythromycin.

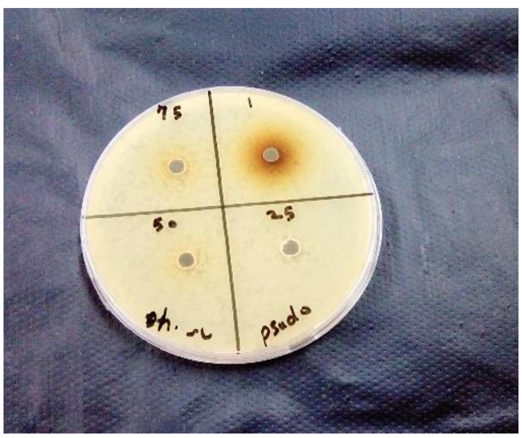

Figure7.The efficacy of *Phlomis syriaca* water extract on *Pseudomonas aeruginosa*

Table 6 Results of Sensitivity and Resistance of Microorganism Strains to Biological Antibiotics, Inhibition Diameter, 1 mm

abbrev. Biological R code	APX	CEC	L	E	PI
conc.	30	30	2	15	20
E. coli	R	R	R	R	6
Pseudomonas aeruginosa	R	R	R	R	9
Klebsiella	R	R	R	R	9
Shigella	R	R	R	R	9
Salmonella	R	R	R	R	8
Staphylococcus aureus	R	R	R	R	9
Listeria	R	R	R	R	20
Candida albicans	R	R	R	R	9

Resistance = R

The gram-positive Listeria bacteria showed high sensitivity to Tobramycin and Pipemidic, with a damping aura of 16 and 20 mm, respectively. The gram-negative Pseudomonas aeruginosa showed good sensitivity against these inhibitors.

The results show that the aura diameters of aqueous extracts were generally larger than the diaphragms of the biological antibiotics used.

CONCLUSION

1. Aquatic extracts of *L. stoechas* and *Boiss* are anti-microbial agents.
2. The aura diameters of inhibition of aqueous extracts were generally greater than the dampening aura diameters of the antibiotics used.
3. Using plant extracts can be a healthy method and technique for preserving meat.

Botanical extracts are considered beneficial nutrients for health because they contain medicinal compounds

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