



## BIOCHEMICAL THEREBY OF MICROORGANISMS CONTAMINATED PRODUCTION LINE FOR SOME PRODUCT FOODS USING *Olea europaea* LEAVES EXTRACT

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### ABSTRACT

The aim of this work was to investigate the antibacterial activity of aqueous and Ethanolic Extracts of Olive Leaves against pathogenic bacteria isolated from raw material, production line and lines and worker swaps samples. These samples were collected from different factories at Dec.2017 to Feb.2018 in egypt. The bacteria were isolated and identified as *E.coli*, *Staphylococcus arueus* and *Salmonella typhimrium*. The dried of leaves olive was extracted by water and ethanol. The antibacterial activity was determined by Disc diffusion assay and Minimum Inhibitory Concentration (MIC) determined. The extracts were found to be effective inhibitory against all the bacterial isolated, but they were effective inhibitory against Gram-positive more than Gram-negative bacteria. The maximum inhibitory zone was noted against *Staph.aureus* (24 mm), *Salmonella typhimrium* (18 mm) and *E.coli* (19.8).The MIC observed from the aqueous and ethanolic leaf extract is 50-60 mg/ml and 30-60 mg/ml respectively for *E.coli*, *Staphylococcus arueus* and *Salmonella typhimrium*.

**Keywords:** Antibacterial activity, Aqueous extracts, ethanolic extracts, *Olea europaea* L.

### INTRODUCTION

Everyone faces the risk of contracting a food-borne illness simply because everyone eats. The World Health Organization (WHO) defines 'Food Safety' as the assurance that, food will not cause

harm to the consumer when prepared and/or eaten in accordance with its intended use. Furthermore 'Food Hygiene' is defined, as all the measures necessary to ensure the safety, soundness and wholesomeness of food at all stages from its production or manufacture until its final consumption (WHO, 2010).

The following microorganisms, *Staphylococcus aureus*, *Salmonella* spp and *Escherichia coli*, and their connection to processing hygiene and food handling practices will be included frequent causes of food poisoning in developing countries. (Marriott and Gravani, 2006).

Now a days, the most commonly used commercial preservatives in the food industry. Although the chemical preservatives, synthetic and semi synthetic, have been widely accepted in the modern era, the undesirable side effects cannot be neglected. With the increasing demand for food safety and health standards, consumers have become more concerned about the presence of chemical residues in the food products. So research on safe plant-derived compounds with antimicrobial activity against foodborne pathogens is vital. Use of natural compounds to reduce foodborne pathogens is gaining popularity worldwide. (Ravishankar et al 2008)

Plant extracts with antimicrobial activity offer a promising alternative to the synthetic preservatives used in food products (Dorman and Deans, 2000).

Recently, people have given more emphasis on the research of such next generation food packag-

ing materials derived from natural plants with antimicrobial activity. Considering the consumers' demand for chemical preservative-free food products, food manufacturers are now using naturally derived antimicrobial agents to replace the traditional chemical ones. There are many advantages to using natural plants as antimicrobial agents: **firstly**, it is cheap especially for use in underdeveloped nations with little access to expensive western medicines; **secondly**, natural spice without any chemical synthetic products should be safer and have fewer side effects; **thirdly**, it is safe to the environment. Thus, it is possible and effective to use these natural plant extracts on food preservation in order to extend the shelf life of products (Abdallah, 2011).

## MATERIALS AND METHODS

### Raw material samples collection

During December 2017 and February 2018, a total of 40 raw material samples (10 sesame, 10 tomato fruits, 10 coca, 10 strawberry), 60 production lines steps samples (10 sesame after wash with tap water, 10 sesame after heat treatment, 10 tomato fruits after wash with tap water, 10 tomato juice after heat treatment, 10 strawberry fruits after wash with tap water, 10 strawberry fruits after heat treatment) and 90 cotton swaps samples from production line and worker (10 sesame production line, 20 worker swap from sesame production line, 10 tomato production line, 20 worker swap from tomato production line, 10 jam production line, 20 worker swap from jam production line) were randomly collected from three different factories in Egypt.

### Purification and identification of bacterial isolates

Bacterial colonies obtained from all cultured on selective media for *E.coli* violet red bile lactose agar, staph.aureus Baird parker agar and Salmonella typhimrium Xylose Lysine Deoxycholate agar were chosen and picked up according to variation in culture characteristics and colony formation then purified by streak-plate method on Nutrient agar medium. Pure isolates were maintained on slants of the same medium at 4°C for subsequent identification. Almost all microscopically examinations and biochemical testing used for identification were carried out according to Bergys' manual (2009).

### Morphological characters

Shape of colony, texture and pigmentation production were examined and recorded.

### Biochemical reaction

Gram reaction, Motility, Catalase, Oxidase, Indole production, Nitrate production, Methyl red, Vogus proskauer, Citrate, Urease, Hydrogen sulphide, Starch and Sugar fermentation were examined and recorded.

### Aqueous extract preparation

Olive leaves (*Olea europea*.L) used in this study were collected from Dept. of Horticulture Fac. of Agric. Ain shams Univ. They were collected in winter (January) and properly prepared for drying process in the day they were collected. The leaves were washed with water to remove impurities such as dust and then dried in an air oven for 3 days at 38°C. The dried leaves were crushed in electrical grinder of powder. Ten grams of powder plant material was extracted in 100 ml of distilled water for 24h at low heat not more than 50°C. then through Whitman No. 1 filter paper and the filtrate was centrifuged at 5000 for 15 min. the supernatant was collected. The procedure was repeated twice and after 6 hour the supernatant was concentrated to make the final volume. The extract was stored at 4°C in air tight bottles.

### Solvent extraction preparation

The extraction was done according to Parekh et al (2005). Ten grams of dried and crushed plant material in electrical grinder were extracted with 100 ml of organic solvent (ethanol) kept on rotatory shaker 190-220 rpm for 24 h, then through Whitman No. 1 filter paper and centrifuged at 5000 for 15 min. the supernatant was collected and the solvent was evaporated at 40°C by water bath to make the final volume one fifth the original volume. It was stored at 4°C in air tight bottles.

### Disc diffusion assay

Impregnated paper discs with crud plant extract were placed on the surface of inoculated agar plates with *E.coli*, *Staph.arueus* and *Salmonella typhimrium* for 24h (4 mm thickness agar layer). The Petri dishes were sealed using para film and left 1h in the refrigerator, in order to allow for the diffusion of the active compounds of the crud plants extracts. Negative controls were done using

## Biochemical thereby of micro organisms contaminated production line for some product 187 foods using *Olea europaea* leaves extract

sterile distilled water instead of active compounds. Then, plates were incubated at 37°C for 24h. The susceptibility of the bacteria to each extract was estimated by measuring the diameter of the zones of inhibition and recorded values as the average of three replicates (NARMS, 2002).

### Minimal inhibitory concentration assay (MIC)

The extracts that showed antibacterial activity were tested to determine the Minimal Inhibitory Concentration (MIC) for bacterial sample.

The isolated bacteria *Staph.aureus*, *E.coli* and *Salmonella typhimrium* were grown in nutrient broth for 24 hr. Then, 200µl of 10<sup>8</sup> cells/ml was inoculated in tubes with nutrient broth supplemented with different concentrations (1 –10 µ/100 µl) of tested extracted.

Afterwards 24 hr a t37°C, the MIC of each sample was determined by measuring the optical density in the spectrophotometer (620nm), comparing the sample readout with that was non inoculated nutrient broth. The MICs were determined as the lowest concentration of tested extracted inhibition visible growth of the lasted culture on the agar plate (Mahdi et al 2013).

## RESULTS AND DESCUTION

### Contaminated bacterial isolates from raw material, production steps and cotton swaps.

Contaminated bacteria isolated from collected samples were summarized in **Table (1)** showed the proportional distribution of foodborne bacterial isolates recovered or found in associated with raw materials, production line and cotton swaps

The total isolates of *E. coli* (87) where (30), (36) and (21) isolates in raw material, production line steps and cotton swaps samples Respectively.

**Table 1.** Incidence of foodborne bacterial isolates from raw material, Production steps and cotton swap samples.

Product type	Bacterial isolates		
	<i>E.coli</i>	<i>Staph.aureus</i>	<i>S.typhiumriu m</i>
Raw material	30/40	30/40	23/40
Production steps	36/60	32/60	27/60
Cotton swaps	21/90	28/90	0/90
<b>Total</b>	<b>87/190</b>	<b>90/190</b>	<b>50/190</b>

*Staph. aureus* (90) isolates were (30), (32) and (28) isolates in raw material, production line steps and cotton swaps samples respectively.

*Salmonella* (50) isolates with (23) and (27) isolates in raw material and production steps and cotton swaps samples respectively.

The results showed that samples contaminated with *staph.aureus* more than *E.coli* and *Salmonella typhimrium*.

### Identification of bacterial isolates

In the present work, the bacterial Isolates contaminated raw material, production line and cotton swaps samples were taken to be identified according to their morphological, cultural characteristics and consumption of broth manual some biochemical tests in (Tables 2, 3) according to (Bergey's manual, 2009).

### Morphological and biochemical identification

The results of morphological and biochemical characteristics of bacterial isolates were given in **Table (2) and Table (3)**. The identified bacterial isolates from all collected samples were belonging to two main bacterial families ( Enterobacteriaceae and Staphylococcaceae).

In the present study the isolated *E.coli* organism fermented mannose, lactose, sucrose and mannitol with the production acid and glucose production both of acid and gas. Results of Methyl Red, Nitrate , Catalase and Indole test of the *E. coli* isolates were positive as reported by (Buxton and Fraser 1977). Also Voges-Proskauer, Coagulase, Oxidase, Urease, Starch hydrolysis, H<sub>2</sub>S production and Citrate utilization test of the *E.coli* were negative. In Gram's staining, the morphology of the isolated bacteria exhibited, small rod shape, smooth and low convex, the motility test were positive and the O<sub>2</sub> requirements were facultative anaerobic. Gram negative bacilli which was supported by several authors (Buxton and Fraser, 1977).

In the present study the isolated *Salmonella typhimrium* organism fermented mannose, glucose and sucrose with the production acid. Results of Methyl Red, Nitrate , Catalase, and H<sub>2</sub>S production test of the *Salmonella* isolates were positive as reported by (WHO, 2007) Also Urease, indole, Coagulase, Voges-Proskauer, Oxidase, , Starch hydrolysis and Citrate utilization test of the *Salmonella* were negative. In Gram's staining, the morphology of the isolated bacteria exhibited, small rod shape, smooth and low convex, the motility test were positive and the O<sub>2</sub> requirements were facultative anaerobic. Gram negative bacilli which was supported by several authors (WHO, 2007).

In the present study the isolated *Staphylococcus aureus* organism fermented mannose, Mannitol, Lactose glucose and sucrose with the production acid. Results of Methyl Red, Nitrate, Catalase and Coagulase test of the *Staphylococcus* isolates were positive as reported by (Habib et al 2015) Also Urease, H<sub>2</sub>S production, Voges-Proskauer, Oxidase, Starch hydrolysis, indole and Citrate utilization test of the *Staphylococcus* were negative. In Gram's staining, the morphology of the isolated bacteria exhibited, small rod shape, smooth and low convex, the motility test were positive and the O<sub>2</sub> requirements were facultative anaerobic. Gram negative bacilli which was supported by several authors ((Habib et al 2014).

**Table 2.** Showed the morphological characteristics of bacterial

Test	<i>E.coli</i>	<i>Staph. aureus</i>	<i>Salmonella</i>
Morphological characteristics			
Shape of colony	Low convex, entire	Raised, circular, entire	Low convex, entire
Texture	Smooth	Smooth	Smooth
Pigmentation	-	Golden yellow	-
Motility	+	-	+
O <sub>2</sub> requirements	F. Anaerobic	F. Anaerobic	F. Anaerobic
Microscopic examination			
Gram reaction	-	+	-
Cell shape	Rods singly or in pairs	Cocci in clusters & pairs	Rods singly or in pairs







F= facultative

**Table 3.** showed the Biochemical characteristics of bacterial isolates.

Test	<i>E.coli</i>	<i>Staph. aureus</i>	<i>Salmonella</i>
Catalase	+	+	+
Coagulase	-	+	-
Oxidase	-	-	-
Urease	-	+	-
Starch hydrolysis	-	-	-
H <sub>2</sub> S production	-	+	+
Hemolysis on blood agar	Gamma	Beta	Alpha
Nitrate reduction	+	+	+
Indole formation	+	-	-
Methyl red	+	+	+
Voges-Proskauer	-	-	-
Citrate utilization	-	+	-
Fermentation of sugar			
D-glucose	A/G	A/-	A/-
Sucrose	-/-	A/-	-/-
Mannose	A/-	A/-	A/-
Lactose	A/-	A/-	-/-
Mannitol	A/-	A/-	-/-

A/G= acid/gas; (+) = positive; (-) =negative

**Table 4.** showed the antimicrobial activity of the crude ethanol plants extract against bacteria isolated.

Olive leaves Extract	<i>E.coli</i>	<i>Staph.aureus</i>	<i>Salmonella</i>
Ethanollic Extract	 21.2±0.36	 24±0.34	 19.85±0.56
Aqueous extract	 21.2±0.36	 24±0.34	 18.33±0.46

#### Anti bacterial activity of used aqueous olive extracts

Aqueous olive leaves extract evaluated for antibacterial activity to inhibit pathogenic bacteria, for find a good alternative used in food industry.

The results in (Table 4) of the clear zones diameter of inhibition for pathogenic bacteria

The data given in Table (4) that, *E.coli* variable sensitivity to the used olive aqueous and ethanollic leaves extracts with average diameter and standard error of inhibition zones, 17.33±0.83 (mm) and 23.33±0.83 (mm) respectively.

The data given in Table (4) that, *staph.aureus* variable sensitivity to the used olive aqueous and ethanollic extracts with average diameter and

**Biochemical thereby of micro organisms contaminated production line for some product 189  
foods using *Olea europaea* leaves extract**

standard error of inhibition zones,  $19.45 \pm 0.54$  (mm) and  $24 \pm 0.34$  (mm) respectively.

The data given in **Table (4)** that, *salmonella typhimurium* variable sensitivity to the used olive aqueous and ethanolic leaves extracts with average diameter and standard error of inhibition zones,  $13.6 \pm 0.83$  (mm) and  $19.85 \pm 0.56$  (mm) respectively.

The olive leaf extracts showed good inhibitory effects on pathogenic bacteria. Many studies confirm positive role of olive leaf in inhibitory pathogenic bacteria. (**Markin et al 2003**) also reported that water extract of olive leaf with a concentration of 0.6% (w/v) killed *E.coli*, *P. aeruginosa*, *S. aureus* and *K. pneumoniae*.

Difference in the phenolic compounds distribution strongly affects the functionality of the extracts such as antimicrobial activity.

Although the individual phenolic compounds in olive leaf extract may show strong in vitro activities, the antioxidant and antimicrobial activities of combined phenolics showed similar or better effects than the individual phenolics (**Lee et al 2010**).

**Turhan (2009)** investigated the effect of different solvents used in extraction on the antimicrobial activity of OLEs against *Staphylococcus aureus* by

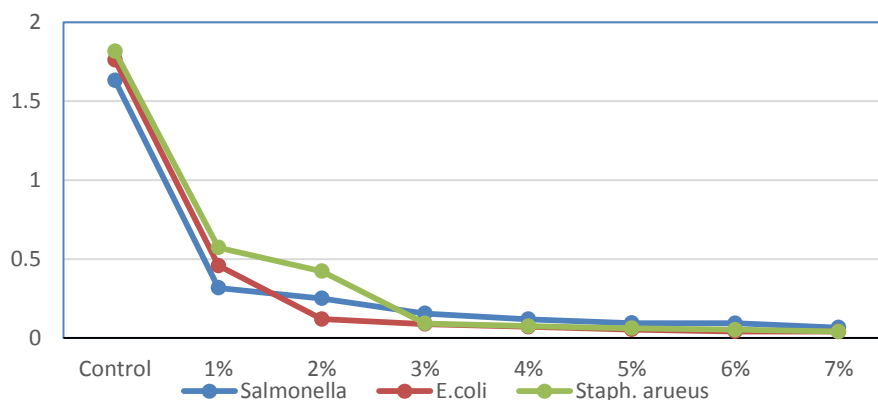
paper disc bioassay. It was reported that the concentration of 129 mg/mL olive leaf water extract showed the highest inhibitory effect on *S.aureus* and followed by chloroform/ethanol and chloroform/methanol extracts.

In other words, flavonoids and phenolic compounds obtained from olive leaf are known to have diverse biological activities and may also be responsible for the pharmacological actions of olive leaf or, at least synergistically reinforcing those actions (**Abaza et al 2007**).

**Minimum Inhibitory Concentrations (MICs) of total Olive leaves extract by spectrophotometer**

The results were illustrated in **Fig. (1)** showed the (MICs) values of aqueous extract of *Olea europaea* the growth of tested contaminated bacteria.

In the presence of aqueous extract of *Olea europaea* at lowest concentration 50 mg/ml got the highest inhibition for *Salmonella* and *staphylococcus arueus*. While *E.coli* required more milligram 60 mg/ml of aqueous extract for highest inhibition.



**Fig. 1.** Curve gram Showing MICs (mg/ml) of aqueous extract of *Olea europaea* against bacterial isolates.

The results were illustrated in **Fig. (2)** showed the (MICs) values of ethanol extract of *Olea europaea* the growth of tested foodborne associated bacteria.

In the presence of ethanol extract of *Olea europaea* at lowest concentration 30 mg/ml and 50 mg/ml got the highest inhibition for *staphylococcus arueus* and *salmonella typhimruim* respective-

ly. While *E.coli* required more milligram 60 mg/ml of ethanol extract for highest

The antibacterial activity was determined by Disc diffusion assay and Minimum Inhibitory Concentration (MIC) and Minimum have been determined. The extracts were found to be effective against all the bacterial strains, but they were effective against Gram-positive more than Gram-negative bacteria

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Lahdibi Sahraoui et al (2017) observed the MIC from the aqueous and methanolic olive leaf extract is 12.5-50 µg/ml and 1.56-12.5 µg/ml respectively.

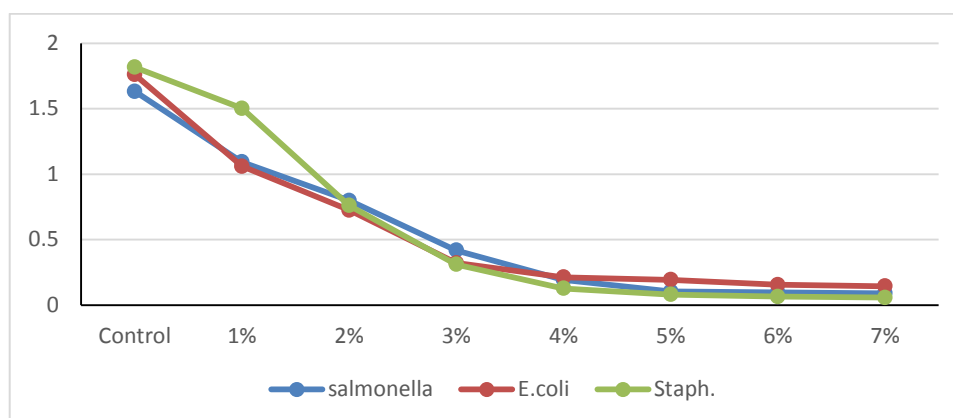


Fig. 2. Curve gram Showing MICs (mg/ml) of ethanol extract of *Olea europaea* against bacterial isolates.

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**Biochemical thereby of micro organisms contaminated production line for some product 191  
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## المعالجة البيوكيميائية للميكروبات الملوثة لخط إنتاج الأغذية المصنعة باستخدام مستخلصات الزيتون

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### الموجز

تركيز قاتل للمستخلص النباتي. أظهر المستخلص النباتي سواء المائي أو الكحولي تأثير ضد الميكروبات المعزولة. ولكن ظهر التأثير علي الميكروبات الموجبة لجرام أكثر من الميكروبات السالبة لجرام. حيث ظهرت أكبر هالة تحلل علي الميكروبات العنقودية بقطر 24 مللي متر وعلي السلامونيلا 18 مللي متر وعلي الايكولاي 19.8 مللي متر. وظهرت النتائج ان افضل تركيز للمستخلصات سواء كان المستخلص المائي او الايثانولي كان من 50 الي 60 مللي جرام و 30 الي 60 مللي جرام علي التوالي وذلك علي جميع الميكروبات المعزولة.

الكلمات الدالة: مضادات البكتريا، المستخلص المائي، المستخلص الايثانولي، الزيتون

يهدف البحث إلى دراسة تأثير مستخلص أوراق الزيتون المائي والإيثانولي كمضادات للبكتريا الممرضة الملوثة لخطوط الإنتاج في بعض المصانع. حيث تم عزل ثلاث انواع من البكتريا من المواد الخام وخطوط الإنتاج والمسحات القطنية للعمال والماكينات. تم سحب العينات من مصانع مختلفة بجمهورية مصر العربية ما بين شهر ديسمبر 2017 إلى شهر فبراير 2018. تم تعريف الميكروبات المعزولة وكانت عبارة عن إيكولاي وسالمونيلا والميكروبات العنقودية. تم عمل مستخلصات ورق الزيتون بواسطة المياه والكحول الإيثانولي. ودراسة التأثير الميكروبي للمستخلصات عن طريق اختبار هالات التحلل لاقراص ورق الترشيح المشبع بالمستخلصات النباتية واختبار دراسة تأثير اقل

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