

A study the effect of alcoholic extracts of rosemary and pome granate plant (*punicagranatum*)on bacterial species contaminating imported chicken thighs in a different markets of of Baquba city

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Abstract

This study was conducted for isolating and diagnosing bacterial species contaminated imported chicken thighs in different market of Baquba city and measuring the effectiveness of inhibitory of the alcoholic extract of rosemary leaves and pomegranate peel against bacterial isolates the study included a collection of (100) samples of chicken thighs from a number of markets located in different parts of Baquba province .

The result showed contamination 80% of samples while 20% showed no microbial contamination , the results of positive specimens and diagnosis of the use of chemical tests have been shown the highest rate of isolation was 50% for bacteria *Salmonella typhimurium* while the lowest rate of isolation of bacteria *Ps.aeruginosa* by 3.75% .

While the results of the distribution of contaminated samples by the company fitted with the origin that the highest percentage of pollution was 26.25% for samples taken from Yumta / Turkey , while the proportion was less isolated was 2.5% to isolate of taken from Houseaf / Turkey , kock / America , Keskinagly/ Turkeycompanies.

The results of distribution of bacterial species according to the company supplied samples showed a ratio of (10,12.5) % for isolates *Salmonella typhimirium*, *E . coli* consecutively of samples processed by the company North chicken / Iraq and by (3.75 , 3.75, 2.5)% for isolates *Ps . aeruginosa*, *Staph . aureus* , *Staph . epidermis* consecutively for samples from yumtal / Turkey company . while the ratio of isolation was 2.5% to *klebsiellaspp* . of samples taken from the two company yumata / TurkeyHouseaf / Turkey for both companies .

The results of the alcoholic extract of the plant of the rosemary leaves and pomegranate peel at the concentration 200mg / ml showed rate of inhibition of (3.5 , 3.8 ,4,6,7,7) mm , (5,5,5,8,8,9) mm in bacteria extracts *Salmonella typhimirum* , *E . coil* , *Ps , aeruginosa* , *Klebsiellaspp* , *Staph epidermis* , *Staph . aureus* consecutively.

key words : *Pomegranate* , rosemary extracts , antioxidant activity

دراسة تأثير المستخلص الكحولي لنباتي أكليل الجبل والرمان على الأنواع البكتيرية الملوثة لأفخاذ الدجاج المستورد في عدد من اسواق مدينة بعقوبة

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المستخلص

أجريت هذه الدراسة لغرض عزل وتشخيص الانواع البكتيرية الملوثة لأفخاذ الدجاج المستورد في عدد من اسواق مدينة بعقوبة وقياس الفعالية التثبيطية للمستخلص الكحولي لأوراق نبات أكليل الجبل وقشور الرمان ضد العزلات البكتيرية، وقد اشتملت الدراسة على جمع (100) عينة من افخاذ الدجاج لعدد من الشركات جمعت من عدداً من الاسواق من مناطق مختلفة في مركز محافظة بعقوبة .

وقد أظهرت النتائج ثلوث 80% من العينات في حين لم تظهر 20% من العينات اي ثلوث ميكروبي وقد بينت نتائج زرع العينات الموجبة وتشخيصها باستعمال الاختبارات الكيموحياتية ان اعلى نسبة عزل بلغت 50% لبكتيريا *Salmonella typhimurium* في حين كانت اقل نسبة عزل بلغت 3.75% لبكتريا *Ps. aeruginosa*

في حين كانت نتائج توزيع العينات الملوثة حسب الشركة المجهزة / المنشأ أن أعلى نسبة ثلوث كانت 26.25% للعينات المأخوذة من شركة yumta / Turkey في حين كانت اقل نسبة عزل بمقدار 2.5% للعينات المأخوذة من شركات keskinagly / Turkey , Kock / America , Houseaf / Turkey وقد اظهرت نتائج توزيع الانواع البكتيرية حسب الشركة المجهزة للعينات نسبة (10 , 12.5) % لعزلات *E. coli* , *Salmonella typhimurium* للعينات المجهزة من شركة North chicken / Iraq ونسبة (2.5 , 3.75) % لعزلات *Staph. aureus* , *Staph. epidermis* للعينات المجهزة من شركة yumta / Turkey في حين كانت نسبة العزل 2.5% من عزلات *klebsiellasp* للعينات المأخوذة من شركتي Houseaf / Turkey وyamata / Turkey وبالتساوي لكلا الشركتين .

وقد اظهرت نتائج اختبار المستخلص الكحولي لأوراق نبات أكليل الجبل وقشور الرمان بتركيز 200mg / ml معدلات اقطار تثبيط بلغت (3.5,3.8,4,6,7,7) ملم (5,5,5,8,8,9) ملم لكلا المستخلصين في بكتريا *Salmonella typhimurium* , *Staph. aureus* , *E. coli* , *Ps. aeruginosa* , *Klebsiellasp* , *Staph. epidermis* على التالي

Introduction

Eating food contaminated with bacteria, parasites and viruses is a major cause of food borne diseases,t typical symptoms include vomiting diarrhea and abdominal cramps (3).

Escherichia coli , *Staphylococcus aureus* , *Salmonella* spp *Salmonella typhimurium* *Listeria monocytogenes* , *Clostridium botulinum* are the main culprits of food borne diseases (6).

The emergence of strains resistant to antibiotics has played a major role in increasing cases of contamination of food with these bacteria(3) recently, the demand for poultry meat has increased, with many characteristics, which are more beneficial in terms of health compared with red meat, prompting producers to increase attention to the quality of poultry meat and the absence of pathogens(12,7).

There are a number of factors that affect the quality of the produced chicken meat, including the personal cleanliness of the workers, the methods of processing, and the characteristics of the meat used in production(7).

In many areas, bacteria contaminated with chicken meat colonize the gastrointestinal tract, including humans, through contamination of meat with these bacteria during the production and delivery stages (26).

Thousands of deaths worldwide are caused annually by salmonella poisoning and typhoid fever, making it a public health problem(5) .

In order to protect consumers from the risk of meat contamination by these pathogens, producers are looking for new ways to reduce the contamination of chicken meat with these bacterial species(9) , and increased consumer preference for original products compared to food with industrial additives (10)It is important to reduce the use of industrial additives including nitrates and replace them with natural compounds such as oils and plant extracts that can be obtained from leaves, fruits, seeds(14).

(25)The use of rosemary oils has demonstrated high inhibitory activity against positive and negative chromosomes. The effect of bacteria in aromatic plants is due to the presence of hydroxyl groups in aromatic plants(29) .

Pomegranate is one of the oldest fruits known and used for eating and increasing fertility has been mentioned in the Bible and the Koran, and the original home of Persia, and spread to Asia and North Africa(24) *punica granatum*l . has been widely used by traditional medicine in American , Asia , Africa and Europe for the treatment of different type of diseases (14,19) .

Punica granatum have been used to treat acidosis , dysentery , microbial infections , pathologies (11).(14)The use of pomegranate extract reduces the growth of *Staphylococcus aureus* growth and subsequent enterotoxin production at 0.01 , 0.05 and 1% u/v concentrations.

(21) Pomegranate has a high antibacterial effect against many bacterial species and this may be due to the possession of many of the antioxidants, which led to increased interest in the extracts of these plants in years has been found that red fruits rich in compounds phenolic antioxidants and anthocyanins (24,13,18).

In bacteria the membrane damage is related to the loss of ions and reduction in membrane potential collapse of the proton pump and ATP depletion . This cytotoxic property is used to treat individuals affected by some human or animal pathogens or parasites (22).

The aim of this study was to isolate and identify gram- negative bacteria that contaminated chicken thighs and the inhibition effect of alcoholic extract of *Rosemary* leaves and *Punica granatum* fruits peels on this bacteria .

Material and method

(100) fresh chicken thighs were collected from different markets of Baquba city.

- Isolation

25grams of chicken thighs were add to 225 ml from nutrient broth media and left for 20 minutes to activate the bacteria , after that attended serial dilution than culture on nutrient agar media , then incubated in 37c°for24 hours .

-Identification

According to (16) we studied phenotypic traits of bacterial isolates include of bacterial colonies size and color on the culture media isolated from it and marginal from , Smell and their surface and also included phenotypic traits and the study of the bacterial cells under oily lens optical microscope after prepared on glass slides to determine in nature of their interaction with gram stain to study the form of cells bacterial grouping made up or not made up spores.

- Preparation rosemary and fruit peels of pomegranate leaf powder .

Rosemary leaves were obtained from the local market and collected pomegranate peels of pomegranate fruits of Diyala province sold in local markets it was dried under normal weather conditions with continuous stirring and milled by blender to obtain a homogenous powder and then save it in glass container while in use .

-Preparation of alcoholic extract cold

100 g of dried vegetable powder was added to 500 ml of ethyl alcoholethyl alcohol concentration of 70 and let it for a period of twenty – four hours at room temperature and then mixed for using filter paper (whatman No . 1) . The evaporation of the solution to rotary evaporator (Rotary Vacuum . Evaporator) temperature 40c while getting a thick liquid where they are dried in the incubator 37 degree during 3 – 4 days and then save the powder in the refrigerator unit use(8) .

- The effectiveness of rosemary and pomegranate fruit peels in the growth of bacteria

Followed diffusion method in agar (Agar diffusion method) as reported in (9) to assess the effectiveness of rosemary extracts and pomegranate against types of bacteria that isolated from chickens thighs by inoculated on Muller Hinton agar with colonies of bacteria isolated by sterile swab from bacterial suspension containing 0.5 cell / ml on McFarland scale than worked wells a diameter of 6 mm on culture media by sterile cork pore and then placed concentration 200 mg / ml in the amount 100 ML with the survival of the control pit containing ethylalcohol with concentration 25% than incubated at 37c° for hours and repeated the experiment three times.

Results&Discussion

Out of (100) samples were collected from the thighs frozen chicken from the local markets for different areas in the center Baquba . contamination was found in (80%)

of samples ratio after cultures and, culture media has been diagnosed with bacterial species according to biochemical test as shown in the table (1)

Table (1) The percentage of the samples

	Identification bacteria	N	%
1	<i>Salmonella typhimurium</i>	40	50
2	<i>Escherichia coli</i>	23	28.75
3	<i>Pseudomonas aeruginosa</i>	3	3.75
4	<i>Klebselliaspp</i>	4	5
5	<i>Staphylococcus aureus</i>	5	6.25
6	<i>Staphylococcus epidermis</i>	5	6.25
7	Positive sample culture	80	80
8	Negative sample culture	20	20
	Total Number	100	100

N: the number of isolates

Distribution of bacterial species varied according to the company processed and origin as shown in the table (2) It has been observed that Yumta / Turkey company have a higher proportion of isolation with ratio 26.25% compared to companies Houseaf / Tuakykock / America , Keskinagly / Turkey that showed less isolated by a ratio 2.5% for both of them The results showed isolated bacteria *Salmonella typhimurium* from most of the brands , the highest percentage of isolation about (12.5 , 10)% to *Salmonella typhimurium*, *E. coli* consecutively while the ratio of isolated (3.75 , 3.75 , 2.5) % to isolates *Ps . aeruginosa* , *Staph aureus* , *Staph epidermis* cosecultively . While the isolation ratio equal between yumta / Turkey and Houseaf / Turkey with ratio 2.5% for both of them.

Table (2) Distribution of bacterial species according to the company processed (origin)

Identified bacteria	N (%)	Yumta / Turkey N (%)	North Chicken / Iraq N (%)	Lezita / Turkey N (%)	Bakpi / Turkey N (%)	Halal / Turkey N (%)	Hastavnk Brazition N (%)	Sadia / America N (%)	Bauvit / Turkey N (%)	Tyson / America N (%)	Houseaf / Turkey N (%)	Kock / America N (%)	Keskinaql yTurkey N (%)
<i>Salmonella typhimirum</i>	40(50%)	7(8.75%)	10(12.75%)	3(3.75%)	3(3.75%)	3(3.75%)	3(3.75%)	3(3.75%)	3(3.75%)	2(2.5%)	0	2(2.5%)	1(1.25 %)
<i>E . coli</i>	23(28.75%)	4(5%)	8(10%)	3(3.75%)	0	0	0	5(6.25%)	0	3(3.75%)	0	0	0
<i>Ps aeruginosa</i>	3(3.75%)	3(3.75%)	0	0	0	0	0	0	0	0	0	0	0
<i>Klebsiellasp p</i>	4 (5%)	2(25%)	0	0	0	0	0	0	0	0	2(2.5%)	0	0
<i>Staph. aureus</i>	5(6.25%)	3(3.75%)	0	0	1(1.25%)	0	0	1(1.25%)	0	0	0	0	0
Staph.epiderm	5(6.25%)	2(2.5%)	1(1.25%)	0	0	0	1(1.25%)	0	0	0	0	0	1(1.25%)
Totalnumber N (%)	80(80%)	21(26.25%)	19(23.75%)	6(7.5%)	4(5%)	3(3.75%)	4(5%)	9(11.25%)	3(3.75%)	5(6.25%)	2(2.5%)	2(2.5%)	2(2.5%)

The results of our study coincided with (28) which reported that the spread of pollution with *Salmonella*, even in the developed countries, the percentage of contamination of these bacteria in Europe was 75%, while 60% in USA, as well as report USDA of the samples contaminated chicken for the period between 2003 – 2005 the pollution by *Salmonella* amount 15,42 % consecutively.

Ps. aeruginosa was less isolate with ratio 3.75%, (28) noted when he studied meat stored refrigerated as increase in the growth of psychrotrophic particularly *pseudomonas* with degree 4c.

The researcher attributed (1) increased pollution of aerobic bacteria species to resistant to different temperature of storage in wholesale stores, as well as misrepresentation of dust and kills addressed and transfer with hand of workers to increased bacterial load of chicken thighs.

The findings show the existence of effective good inhibition of alcoholic extract of rosemary leaves and peels of pomegranate as it was the inhibition diameter rates for both extracts about (3.5, 3.8, 4, 6, 7, 7), (5, 5, 5, 8, 8, 9) mm in *Salmonella typhimurum*, *E. coli*, *Klebsiella spp*, *Ps. aeruginosa*, *Staph. epidermis*, *Staph. aureus* respectively as shown in Figure (3)

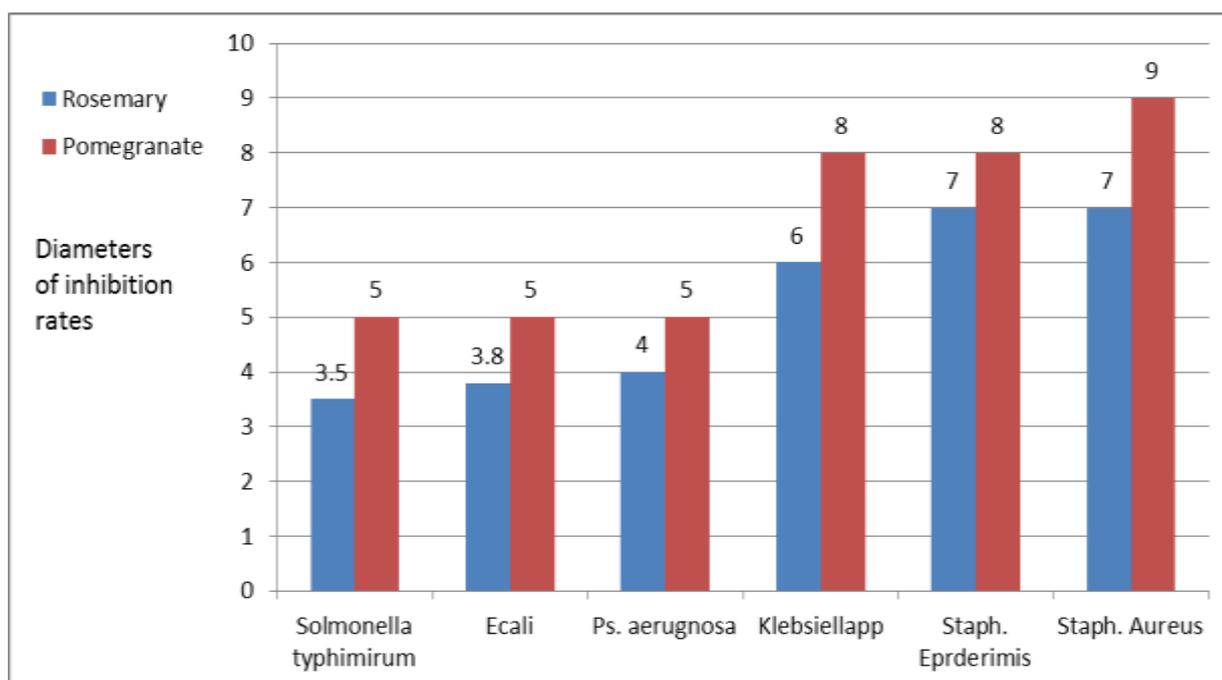


Figure (3) Inhibitory effect of rosemary and pomegranate plants against bacteria species isolates from chicken thighs

It has interpreted (22) The presence of carnosic acid in the rosemary extract gives it a high anti-microbial effect, especially against the Gram-positive bacteria, which is more effective than the rosemarnic acid found in the rosemary extract, The best antibacterial activity determined by the both micro dilution method was seen against *B. cereus* and the lowest activity against *S. infantis* (18)

Punica granatum has high anti-microbial activity against tested isolates, these results were in agreement with the results of (27) who found that extracts of *Punica granatum* has a strong antimicrobial.

(26) found that different extracts of *Punica granatum* give good antibacterial activity against different bacterial strains, this is due to the presence of metabolic toxins and anti-bacterial compounds are very effective (20,32)

While another study reported that *Punica granatum* contain large amount of tannins 25% and the antibacterial activity may be indicating presence of some secondary metabolites(31).

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