The antibacterial activity of alcoholic extract of oak against some yolk sac infection produced by bacteria in broiler chicks

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Abstract

The present study aimed to investigate the antibacterial activity of the graduated concentrations from the alcoholic extract of oak (*Quercus acuta*). (25, 50, 100, 200 mg/ml) were tested against the growth and multiplication of some pathogenic bacteria causing yolk sac infection in broiler chicks (*S. aureus*, *Streptococcus spp*., *Salmonella spp.* and *E. coli*) were compared with the effectiveness of ampicillin (10µg), gentamicin (30 µg) and neomycin (30 µg) in culture media, in the same time we also investigate the effect of incubated temperature on the growth inhibition zone of the oak extract through conservation of culture media in two different temperature (25°, 37°C) for 24 hrs with the helping of incubator. The result revealed that the mentioned bacteria showed high sensitivity toward different concentrations of alcoholic extract of oak and this result were close, often, especially at high concentrations. Our result revealed that the activity increased with increase concentration of extract, in the same time we observed that the incubation temperature factor showed significant effects on the results especially with the growth of *salmonella* and *E. coli* in culture media.

الفاعلية المضادة للجراثيم للخلاصة الكحولية لنبات الجفت ضد بعض الجراثيم المحدثة لإصابات كيس المح في أفراخ دجاج اللحم

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الخلاصة

هدفت الدراسة الحالية التحري عن النشاط المضاد للجراثيم لتراكيز متدرجة من الخلاصة الكحولية لمادة الجفت وهي (25، 50، 100، 200 ملغم/ مل) وقد اختبرت ضد نمو وتكاثر عدد من مسببات إصابات كيس المح في أفراخ دجاج اللحم وهي العنقوديات الذهبية، المسبحيات، السالمونيلا والاشريشيا القولونية وثم مقارنتها مع فعالية المضادات الحيوية الامبسيلين (10 مايكروغرام/ مل) ، الجنتامايسين (30 مايكروغرام/ مل) والنيومايسين (30 مايكروغرام/ مل) في الأطباق الزرعية. وتم التحري أيضا عن تأثير درجة الحرارة 25°م و37 °م مدة 24 ساعة بمساعدة الحاضنة. بينت نتائج الدراسة إن الجراثيم المذكورة أظهرت حساسية عالية تجاه التراكيز المختلفة للخلاصة الكحولية لنبات الجفت وكانت النتائج متقاربة في الغالب وخصوصا عند التراكيز العالية، كما كان لعامل التركيز تأثيراً واضحاً إذ إن زيادة التركيز رافقت زيادة في أقطار تثبيت النمو، في الوقت نفسه كان لعامل حفظ الأطباق الزرعية في درجات حضن مختلفة (25°، 37°م) هو الآخر تأثيراً معنويا على النتائج خصوصاً على نمو جرثومتي السالمونيلا والاشيريشيا القولونية في الأطباق الزرعية.

Introduction

Yolk sac infection is an economically important disease since it increases first week mortality and causes poor weight gain. In addition, birds that survive to yolk sac infection show poor carcass quality (1,2). Yolk sac infection can occur in all flocks

resulting in decreased hatchability, increased mortality and increased cull rate due to retarded growth (3, 4). Yolk sac infection occurs mainly due to bacterial contamination of the egg-shell at the broiler breeder farm, shortly after the egg is laid, while the cuticle is still moistened (4,5). Different type of bacterial agents are attributed for causing yolk sac infection. *Escherichia coli* was frequently the main one involved. Its isolated from yolk sac infection was reported by (6,7,8). In addition bacteria of the genus *salmonella* was reported by (9,10,11). *Staphylococcus, Streptococcus, Pseudomonas spp., Enterobacter spp., Klebsiella spp., clostridium spp., Enterococcus spp*. that have been isolated from yolk sac infection in chicks in different location all over the world (6,8,9). The contamination takes places due to several factors including lack of hygiene in nests, presence of eggs on floor, incubation of dirty eggs or eggs with egg-shell defects and collection of dirty and clean eggs together (3,4). New antimicrobial agents are needed to treat diseased condition in humans and animals caused by drug resistance microorganisms, in addition, there is a continuing consumer demand for "natural" and/ or preservative-free microbiologically safe foods and cosmetic products (12). Focus on plant research has increased all over the world and large body of evidence has collected to show immune potential of medicinal plants used for their therapeutic abilities against micro-organisms. New trend of research concentrating on preparing plant extracts has been established for producing medicinal drugs based on scientific criteria. Besides, these extracts overcome the harmful effects encountered with the use of antibiotics and the resistance of micro-organisms to the antibiotics. The mechanisms by which plant extracts control bacterial activity take many routes (13). Antimicrobial compounds of plants origin may occur in stems, roots, leaves, bark, flowers and fruits of plants (14) most of these parts contain many active compounds, consequently they are multipurpose at the same time (15). Among these compounds are simple phenols in which their activity come from the presence of hydroxyl groups (16) other compounds isolated from different plants such as flavonoids, tannins, quinines, coumarins and terpenoids had been extracted and found to be of inhibitory effect on numerous bacterial strains as well as fungi (17). The present study aimed to investigate antibacterial activity of ethanolic extract of oak against growth of some pathogenic bacterial isolates from yolk sac infection in broiler chicks under two incubated temperature (25°,37°C) in culture media.

Material and Methods

- **Plant preparation and extraction:** Dry oak (*Quercus acuta*) were purchased from a local market in Al-Diwanyia city and were identified in the national Iraqi institute for herbs. Then grinded to very well until it became as a fine powder. fifty gm of oak powder were soaked in 125 ml of 96% ethanol. This mixture was then stirred for 24 hrs at 30°C and filtered using whattman No.3 filter paper. finally, the filtrate was evaporated by using rotary evaporator (40-60° C) until fully dried.
- **Preparation of test extract solutions:** A series of different oak extract concentration (25, 50, 100, 200 mg/ml) were prepared by dissolving a known weight of plant extract in 25% (v/v) ethanol which acted as a solvent.
- **Micro-organisms:** In the present study two gram positive bacteria (*S. aureus, Streptococcus spp*.) and two gram negative bacteria (*Salmonella spp.* and *E. coli*) were tested which were obtained from educational veterinary hospital in Al-Diwanyia which isolated from chicks suffering from signs of yolk sac infection. The bacteria have been diagnosed according to (18) and (19) and maintained on nutrient agar slants.

- **Antibacterial assays:** The agar well diffusion was prepared by adding 0.1 ml of 1×105 cfu from each bacteria in to the mauler Hinton agar in volumic flask and homogenized slowly for a few seconds. The mixture was then poured into a Petri dish and allowed to solidify prior to the preparation of 6 mm diameter wells made by using a sterilized pasture pipette. 0.1ml of oak extract solution in different concentrations were transferred in to each well allowed to set. standard disc of ampicillin 10µg, gentamicin 30 µg and neomycin 30 µg obtained from oxoid (LTD) and served as a positive control while use ethanol solution 25% as a negative control for antimicrobial activity. The plates were incubated at 25°c and 37°c and the diameter of inhibition zone surrounding each well and disc were measured by mm.

Result and Discussion

yolk sac infection (YSI) is considered a major cause of mortality in broilers during their first week of life (3, 20, 21). Yolk retention and yolk sac infection is considered as an important cause of death in chicken. Yolk sac infection is not the only cause of death in chicken but also in other species of poultry including guinea fowl, duck, turkey, quail and goose. It was reported as the most frequent cause of death in indigenous guinea fowl (22). With the increasing occurrence of bacterial resistance changes in the concentration of the test material against available antibiotics, it has become essential to look for new antibiotics. Most of the antibiotics available today come from natural origin, especially from various microbial or plant sources. Higher plants also produce compounds to protect themselves from microbial attacks. For screening antimicrobial properties of plant extracts used agar plate techniques world wide (23). *Quercus* (oak) bark and galls are used as an astringent, antiseptic and hemostatic. A decoction of *Quercus* is also used to treat acute diarrhea and inflammation. Moreover, the decoction of these plants could be used for burns and cuts (24). Agar well diffusion methods are widely used because of their simplicity in hand and low cost in the other hand, in addition to that it may help to detect if there is any resistance from the bacteria to any drug, medicinal plant or agents that may be used to study it is effect (25). In this study the antimicrobial activity of different concentrations of ethanolic extract of oak against a number of gram positive bacteria (*S. aureus*, *Streptococus spp*) and gram negative bacteria (*Salmonella spp* and *E.coli*) that isolated from yolk sac infection in broiler chicks was determined by agar-well diffusion method. Results of the antibacterial activity of ethanolic extract of *oak* against tested microorganisms at two different incubated temperature (25°, 37° C) are listed in Tables (1-2). According to this results, the ethanolic extract of oak at all concentrations used in the present study was active against gram positive and negative bacteria with variation of this result according to the three factor, the first one was concentration of oak extract, the second one was the type of bacteria, the third one was the degree of incubated temperature of culture dishes (25°,37°C) this result support other studies in this aspect. Serit *et al* (26) concluded that ethanolic extract of *Querus acuta* trunk have antibacterial activity against both gram positive and gram negative bacteria in culture media. The results showed that there was a proportional relationship between the concentrations of the oak extracts that had been used and the zone of growth inhibition of all tested bacteria at two incubating temperature (25°,37°C) on the Moeller Hinton agar this mean that increase in concentration of extract accompanied by increased in the zone of growth inhibition of all of the microorganisms and this may be due to the increase in concentration of extract accompanied by increase in the active ingredient (or ingredients) which are present in the ethanolic extract. For the detection of oak activity there was a significant differences in the inhibitory effect (at $P<0.05$) between different concentrations on the same

bacteria and between the concentrations of extract and positive control (standard antibiotics) and negative control (ethanol) in the other hand. The ethanolic extract of oak at various concentrations showed a variety of antibacterial activities against four type of bacteria the highest zone of growth inhibition was exhibited by the extract at 200mg/ml was on *E.coli* giving a zone diameter with standard error (SR) (22±1.73 mm) at 25°C incubation temperature and 20.66±0.57mm at 37°C when administered at the same concentration. The lowest zone of growth inhibition was observed against *Salmonella spp* giving a zone diameter with SR (11.33±0.57 mm) (7.33±0.57 mm) for dishes incubated at 25°, 37°C respectively at 25 mg/ml. The antibacterial effectiveness of the oak ethanolic extract may due to the synergistic effect of a number of compound which are present in this plant especially to the tannins and polyphenolic compounds (27). Several esters of gallic acid as n-propyl gallate, n-octyl gallate and n-dodecyl gallate have shown antibacterial effectiveness inside antioxidant activity (28). Gallic acid which is considered one of the component of the extract have no activity against bacteria, although it was previously reported to have inhibitory activity against *E.coli*. (27, 28), the presence of antibacterial substances in the ethanolic extract of *Q.acuta* as compound II (4,5-Di-o-galloyl (+) –protoquercitol) and compound III (3,5-Di-o-galloyl (+)-protoquercitol) correspond with some reports on the antibacterial and antifungal activities of several species of quercus (29), the reported presence of various quercitol gallates in *quercus spp*. which are possibly to have anti- bacterial makes it logical to suspect that liquors which had previously been matured in oak might contain some antibacterially active gallates (30). Although the effect of oak on the growth and multiplication of number of gram positive and negative bacteria *in vitro* in local study is promising, further microbiological, pharmacological and clinical trials are required.

Table (1) The effect of oak extract in different concentrations and some antibiotics against growth of some pathogenic bacteria under 25°c incubator temperature

extract concentration (mg/ml)	Type of bacteria and zone of inhibition (mm)			
	S. aureus	*Streptococcus spp.*	*Salmonella spp.*	*E. coli*
25	12± 0[aA]	11.33± 0 [aA]	11.33± 0 [aA]	13.66± 0.57[aA]
50	12.33± 0.57 [aA]	14.66± 0.57 [bB]	14.33± 0.57 [bB]	17± 0 [cB]
100	14.33± 0.57 [aB]	19 ± 1 [bC]	16.66± 0.57 [cC]	19± 0 [bC]
200	19.33± 0.57 [aC]	21.66± 0.57 [bD]	21 ± 0 [bD]	22±1.73 [bD]
Ampicilin (10µg)	R[aD]	12± 0 [bA]	R [aE]	R [aE]
Gentamicin (30µg)	R[aD]	R [aE]	15 ± 0 [bB]	16.66± 0.33 [bB]
Neomycin (30µg)	16± 0[aE]	15.33± 0.66 [aB]	15.66± 0.33 [aB]	19± 0 [bC]
Ethanol (25%)	0 ± 0[aE]	0 ± 0 [aE]	0 ± 0 [aE]	0 ± 0 [aE]

- **Different capital letters mean significant differences for vertical values at level (p<0.05).**
- **Different small letters mean significant differences for horizontal values at level (p<0.05).**
- **Result for 3 isolates for each bacteria with SE.**
- **{R} means resistance of bacteria to antibiotic.**

Table (2) The effect of oak extract in different concentrations and some antibiotics against growth of some pathogenic bacteria under 37°c incubator temperature

extract concentration (mg/ml)	Type of bacteria and zone of inhibition (mm)			
	S. aureus	*Streptococcus spp.*	*Salmonella spp.*	*E. coli*
25	12± 1.73[aA]	10.66± 0.57[aA]	7.33± 0.57[bA]	9± 0 [bA]
50	13.6±1.15 [aA]	13.66± 0.57 [aB]	9.33± 0.57 [bA]	13.3± 0.57 [aB]
100	14.33±1.15 [aAB]	19.66± 0.57 [bC]	11.66± 0.57 [cAB]	17± 0 [dC]
200	19.33± 1.15 [aC]	20 ± 0 [aC]	14.66 ± 0.57 [bC]	20.66± 0.57 [aD]
Ampicilin (10µg)	R[aD]	12± 0[bA]	R[aD]	R[aE]
Gentamicin (30µg)	R[aD]	R[aD]	15.66 ± 0.33 [bC]	17± 0 [bC]
Neomycin (30µg)	16± 0[aB]	15.33± 0.66 [aB]	15.66± 0.33 [aC]	18.33±1.15 [bCD]
Ethanol (25%)	0 ± 0[aD]	0 ± 0[aD]	0 ± 0 [aD]	0 ± 0 [aE]

- **Different capital letters mean significant differences for vertical values at level ($p<0.05$).**
- **Different small letters mean significant differences for horizontal values at level ($p<0.05$).**
- **Result for 3 isolates for each bacteria with SE.**
- **{R} means resistance of bacteria to antibiotic.**

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