Effect of *syzygium aromaticum* and *commiphora myrrha* extracts on broiler chickens infected with *E.coli*

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Abstract

Forty-nine *E. coli* isolates were isolated from 80 broiler chicks collected from broiler farms. These isolates were serotyped into O1, O8, O18, O78, O86, O111 and untyped (17 serotypes). The growth inhibitory effect of *Syzygium aromaticum* (clove) and *Commiphora* myrrha (myrrh) on *E. coli*. Aquous, 80% ethanol and n-hexan plant extracts were tested against *E. coli*. Agar well diffusion method, minimum inhibitory concentration (MIC) values were used in these investigation. The findings indicated that *S. aromaticum* and *myrrh* had grow inhibitory effect against tested *E. coli*. Tow hundred one day old Ross chicks were divided into 5 equal group (1, 2, 3, 4 and 5), group (1) chicks remained a negative control. Groups (2, 3 and 4) chicks infected by *E. coli* O_{111}. Group (3) chicks were treated with 25mg/L clove and group (4) treated with 25 mg/L myrrh for 3 successive days. The clinical signs, mortality rate, postmortem lesion reisolation of *E. coli*, body weight gain and feed conversion rate (F.C.R) after two week of infection were recorded. The clove extracts were more effective for controlling the *E. coli* than myrrh. It could be concluded that *S. aromaticum* (clove) and commiphora (myrrh) extract had grow inhibitory against tested *E. coli*, and clove has superior activity and efficacy than the myrrh in treatment of broiler chicks infected with *E. coli*. Also, the simultaneous use of both *S. aromaticum* (clove) and commiphora (myrrh) extract give synergestic effect in treatment of broiler chicks infected with *E. coli*.

Key words:
Syzygium aromaticum, commiphora myrrha, *E.coli*

Introduction

Bacterial infectious diseases represent an important cause of morbidity and mortality worldwide. An antibiotic resistant bacterium is a threat which is becoming increasingly common **(Lesse, 1995).**

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs **(Gislene *et al.*, 2000 and Cos *et al.*, 2006).**

Plants are rich source of natural products used for centuries to cure various diseases. The plant-derived medicines are based upon the premise that they contain natural substances that can promote health and alleviate illness.

So, a return to natural substances are an absolute need of our time **(Swayamjot *et al.,* 2005 and Kumar *et al.,* 2007)**.

Several studies have demonstrated potent antibacterial effects of *clove* **(Lopez *et al.,* 2005; Li et al, 2005; Betoni et al, 2006 and Fu et al, 2007).** The inhibitory activity of *clove (Syzygium aromaticum)* is due to the presence of several constituents, mainly eugenol, eugenyl acetate, betacaryophyllene, 2-heptanone **(Chaieb et al , 2007)**, acetyl-eugenol, alphahumulene, methyl salicylate, iso -eugenol, and methyl-eugenol **(Yang *et al.,* 2003)**.

Commiphora molmol (myrrh) is widely distributed in the Kingdom of Saudi Arabia and it is grown in Jizan area on Red Sea coast. It is also found in Somalia and other coast African countries **(Mugahid, 1981).**

It is used in traditional medicine as antiseptic, carminative, anti-inflammatory, **(Tarig *et al.,* 1985).** The ethanolic extract of *C. molmol* exhibited antimicrobial activity against the Gram negative organisms ***(*Omer *et al.,* 2011).**

The development of new antimicrobial agents for the treatment of bacterial infection is of increasing interest. In the last few years a number of studies have been conducted to verify the effectiveness of plant extracts against bacterial infections **(Prashanth *et al.,* 2006 and Ung *et al.,* 2010).**

The present study evaluated the individual and in combination growth inhibitory effect against enterobactrecea.

Material and Methods

Eighty diseased broiler chicken aging one day up to 36 days were used in this investigation. The examined chicks were suffering from sever respiratory manifestation and collected from broiler farms.

Bacteriological examination.

Specimens from heart blood, liver, spleen, unabsorbed yo1k sac and air sacs were cultured on MacConkey and blood agar, incubated at 37^{o}C for 24 hrs for detection of *E. coli*. The suspected colonies were identified morphologically as well as biochemically according to **(Kstman *et al.,* 1996)**.

Serological identification.

Antisera of *E. coli* was used for serological identification of somatic antigen (O) using slide agglutination test according to **(Edward and Ewing, 1972)**.

N.B. Antisera of *E. coli* were obtained from Denka Sicken Co. Ltd, Tokyo, Japan.

Experimental chicks.

Two hundred, one day-old Ross broiler chicks were obtained from Socary Hatchery and feed on balanced ration free from medication.

Plant material and extraction.

The two natural plant samples used in this study were flowers of *Syzygium aromaticum* (clove) and unorganized part of *Commiphora myrrha* (myrrh).

Samples were purchased from market, distributed in Zagazig region during April 2014, then dried in an incubator at 37^{o}C, the samples were ground into fine powders using electric blender and extracts were by soaking 125mg of each samples separately in 500ml solvents, (distilled water 80% ethanol and n-hexan) using conical flasks plugged with cotton plugs, the mixture were kept at room temperature for 72hrs under discontinuous shaking. The crude extracts were filtered through sintered glass funnel (500 ml) under vacuum. The filtrate were evaporate to dryness by rota-vapour (Buchi, R-215, Switzerland), the rotary water bath was adjusted to 0^{o}C, then the extract were kept over-night under vacuum fume hood to obtained a constant dry weight and extracts stored at 4^{o}C for further use. The extracts weighted and dissolved at concentration of 50mg/ml.

Antibacterial tests.

Independent and in combination water, 80% ethanol and in-hexan extracts of tow plants were tested against *E. coli* O_{111}. The growth inhibitory affect were determined by agar well diffusion method as previously described by **(Collins *et al.,* 1995)**. Active cultured for experiments were prepared by transferring loopful from *E. coli* O_{111} to test tubes of Mueller-Hinton broth (Oxoid – England). The test plates were incubated at 37^{o}C for 24 hrs., the cultured were diluted with broth to achieve an

optical density corresponding to 2.0 X 10^6 (cfu/ml) after agar solidification. Muller Hinton agar was swapped with suspension of *E. coli* O_{111} using sterile cotton swab. The medium was punched with six millimeters diameter well, and filled 100 ml of test sample and allowed to diffuse at room temperature for 20 mint.

The minimum inhibitory concentration (MIC) of individual and in combination extract were determined by micro dilution technique as described by National Committee for Clinical Laboratory Standard **(NCCL, 2000)**.

Experimental infection.

Two hundred day old Ross chicks were divided into five equal groups (1, 2, 3, 4 and 5) of 40 chicks each were reared in separate unit and fed on ration without any medication. Group (1) chicks were kept as negative control (non infect and non treated). At 7 day-old group 2, 3 and 4 chicks were injected with *E. coli* O_{111} in a dose 0.25 ml of 2 x 10^6 cfu/ml intratracheal injection according to **(Stipkovits, 1988)**. Group (2) chicks, were remained as positive control, group (3) chicks were treated with a dose 25mg/L of *Syzygium aromaticum* extract in drinking water for 3 successive days. Group (4) chicks were treated with a dose 25mg/L of myrrh extract in drinking water for 3 successive days.

Group (5) chicks were treated with 25mg/L of both S. *aromaticum* and myrrh extract in drinking water for 3 successive days.

Two weeks post treatment all remaining chicken were weighed. The body weight gain, food consumption (F.C) and feed conversion rate (FCR) were determined. 5 chicken of each group were sacrificed for P.M and bacteriological examination.

Results

Bacteriological isolation and serological identification.

The results of bacteriological examination of 80 diseased chickens for detection of *E. coli* revealed 49 strain of *E. coli* were isolated. Serogrouping of the isolates indicates presence 6 different serotype and 17 strains were untyped.

Table (1). Serological identification of pathogenic avian *E. coli* (49 strain) isolated from broiler chicks suffering from respiratory disease.

Serogroup	O1	O8	O18	O78	O86	O111	Untyped strains
Number of isolates	5	2	3	6	5	11	17
%	10	4.6	6.1	12.2	10	22.4	34.6

The inhibitory effect of aqueous, 80% ethanol and n-hexane plant extracts (alone or in combination) was evaluated on *E. coli*. The result of inhibition zone diameter (IZD) and MIC of independent plant extracts against *E. coli* waere determined. Aqueous extract of clove exhibited inhibitory effect against *E. coli* with IZD 14.3 mm and MIC 3.12 mg/ml, while *E. coli* were susceptible to ethanolic and hexanic extract of *S. aromaticum* 17- mm and MIC (6.12 mg/ml).

The ethalonic extract of *Commiphora myrrha* showed IZD of 14.33 mm and MIC of 6.52 mg/ml while its aqueous extract did not inhibit *E. coli*.

Experimental infection.

Clinical signs, mortality rate and lesion score in infected non treated birds group showed sign of depression off food, diarrhea with sever P.M. lesion (air saculits, pericarditis and perihepatitis) and high mortality reach 60%. These finding was subsided post treatment with both extracts and the mortality rate was 15% with mild lesion score with *S.aromaticum* while the morality rate was 20% in chicken treated with *Commiphora myrrha*.

All chicks of group (l and 5) were negative culture for *E. coli* (O111), however, a higher frequency of resolution of the pathogen from liver and heart of group 2 compared with those

groups 3 and 4 for *E. coli* symptoms were observed.

The influence of infections by *E. coli* body weight, food consumption (F.C) and feed conversion rate (FCR).

The mean body weight of infected, untreated chicks was significantly ($p>0.05$) decrease than infected and treated chicks (table 3).

Table (2). Mortality rate lesion score reisolation of *E. coli* O111 isolates following and treatment *S. aromticum* and *Commiphora myrrha* extracts 25mg/ml for 3 successive days.

Parameter / Group	Mortality %	Lesion score			Frequency reisolation
		Air saculities	Pericarditis	Perihepatitis	
1	0	0	0	0	0
2	60	70	70	70	75
3	15	20	20	20	20
4	20	25	25	25	25
5	0	0	0	0	0

Table (3). The influence of *S. aromticum* and *Commiphora myrrha* extract 25mg/ml for 3 day on experimental chicken performance

Group	Befor treatment 10th day infection				Two week post treatment			
	Body weight (g)	Body weigh gain	F.C	FCR	Body weight (g)	Body weigh gain	F.C	FCR
1	230	190	340	1.57	1100^{b}	870^{b}	1449^{b}	1.7
2	236 ± 12	195 ± 7	307 ± 15	1.57	820^{d} ± 38	584^{d} ± 32	1226^{d} ± 42	2.1
3	238 ± 12	195 ± 8	303 ± 12	1.55	970^{c} ± 20	732^{c} ± 21	1318^{c} ± 40	1.8
4	236 ± 16	195 ± 12	308 ± 8	1.58	940^{c} ± 25	704^{c} ± 26	1302^{c} ± 38	1.85
5	232	191	299.9	1.57	1200^{a} ± 30	960^{a} ± 32	1598^{a} ± 41	1.65

Different letters in the same column significant difference ($P\leq 0.05$).

Discussion

Plants remain one of the main sources of the natural products for new therapies particularly in poor countries because most of them are cost less, affect a wide range of antibiotic resistant microorganism and another reason is there is an erroneous impression that herbal medicines have fewer adverse effects. In these study extraction was done using water 80% ethanol and n-hexan. Chemical content of plant extracts differ depending on the nature of the solvent used in extraction procedure **(Jules *et al.*, 2011)**.

In the present study individual ethalonic and n-hexanic extracts of clove showed inhibitory activity against *E. coli*. Our finding agree with other observations **(Sulieman *et al.*, 2007 and Ram *et al.*, 2010)** who demonstrated the clove ethanolic extracts exhibited the maximum zone of inhibition against tested bacteria. Variable grow inhibitory effect of individual water, ethalonic and hexanic extracts of myrrh on

tested bacteria was detected with IZD ranged from zero – 14.3mm. This finding was supported by **(Omer *et al.*, 2011)**.

Experimentally infected chicks with isolated *E. coli* O111 were showed clinical signs in form of sever respiratory signs with air saculits, pericarditis and perihepatitis on postmortem examination. Experimentally infected chickens showed high mortality rate (60%). The mortality rate infected chickens was reduced to 15 and 20% after treatment with *Syzygium. aromticum* and *Commiphora myrrha* extracts respectively.

The treatment with *Syzygium. aromticum* was more effective than *Commiphora myrrha* for controlling infection *E. coli* supporting it is still effective lead to decreasing mortality from 60% (infected and non-treated) to 15%, this may be due to *Syzygium aromticum* extract active against *E. coli*. The same result of **(Ram *et al.*, 2010)** who demonstrated clove extract exhibited the maximum zone of inhibition against tested bacteria.

Commiphora myrrha extracts has low efficacy for controlling *E. coli* infection of chicks with high lesion scores and high mortality rate.

Also improvement of general condition body weight gain and feed conversion rate caused by treated with *Syzygium aromticum* and *Commiphora myrrha* were recorded after use of both extracts in this study **(Ferendez *et al.*, 1998)** stated that the improvement of body weight gain in infected bacteriological effect of antibiotics on the infection and consequently improvement of general health condition.

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تأثير مستخلصات القرنفل والمر علي بداري التسمين المصابة بالإيشيريشيا كولاي

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الملخص العربى

يهدف هذا البحث لتقييم استخدام القرنفل والمر لدجاج التسمين بمرض الايشريشيا كولاي ، ولهذا تم عزل 49 عترة من الميكروب القولوني العصوى من عدد 80 كتكوت تسمين مريضة بمشاكل تنفسية، وبإجراء الاختبارات السيرولوجية وجد 6 عترات من هذا الميكروب تم التعرف عليها وأخرى لم يتم التعرف عليها ، كما تم تقييم التأثير المثبط لمستخلصات نباتي القرنفل والمر علي بكتريا الإيشيريشيا كولاي.

أظهرت هذه النتائج أن مستخلصات القرنفل والمر لها أثر مثبط علي نمو بكتريا الإيشيريشيا كولاي، ولكن مستخلص القرنفل الإيثيلي له الأثر الأكبر كمثبط لنمو الإيشيريشيا كولاي.

ولمعرفة فاعلية هذه المستخلصات علي الدواجن تم تقسيم 200 كتكوت تسمين روش عمر يوم - خمسة مجاميع متساوية - وتم عمل عدوى بميكروب الإيشيريشيا كولاي (O111) عمر 7 أيام وتركت المجموعة الثانية دون علاج وتم علاج المجموعة الثالثة بمستخلص القرنفل 25مجم/لتر لمدة ثلاثة أيام والمجموعة الرابعة بالمر 25مجم/لتر لمدة ثلاثة أيام والمجموعة الخامسة تم علاجها بمستخلص القرنفل 25مجم/ لتر و مستخلص المر 25مجم/لتر.

واستناداً إلي الأعراض النفسية ونسبة النفوق، الصفة التشريحية وإعادة عزل الميكروب ومقارنتها بالدجاج المصاب والمعالج.

بالإضافة إلي الوزن المكتسب ونسبة التحويل الغذائي في كل المجاميع بعد نهاية الأسبوع الثاني يمكن التغلب علي مشاكل الإيشيريشيا كولاي باستخدام مستخلص القرنفل أفضل من استخدام المر كما أن العلاج بهما معا يعطي نتائج أفضل من استخدام اي منهما منفردا.