



## Antibacterial activity of some medicinal plant oils against *Escherichia coli* and *Salmonella* species in -vitro

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### ABSTRACT

A total of 100 samples were collected (75 samples from diseased broiler chickens and 25 samples from recently dead broiler chickens). The samples were examined bacteriologically, fifty two isolates of *E.coli* (52%) and seven isolates of *Salmonellae* (7%) were found. ten random samples of *E.coli* were sero grouped where five were *E.coli* O78, two O157 and untyped and seven isolates of *salmonellae* were serotyped where three *S.Enteritidis*, one *S.Charity*, one *S.Remiremont* and two untyped . Antibacterial activity of five medicinal plant oils from Eucalyptus, Mint, Cinnamon, Garlic and Thyme were evaluated against the isolated strains using micro-titer plate to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for these oils. MIC results proved that for Mint, Cinnamon, and Garlic oils were ranged from 5-5120 µg/ml, for Eucalyptus 20-5120 µg/ml and for Thyme 5-2560 µg/ml. While results of MBC of Thyme and Cinnamon oils were ranged from 10-2560 µg/ml. but MBC of Eucalyptus ranged from 40-5120 µg, MBC of Mint and Garlic oils were ranged from 5-2560µg |ml, 20-2560µg |ml respectively.

**Keywords:** *E.coli*, *Salmonella*, Essential oils, MIC, MBC

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### 1.INTRODUCTION

*E. coli* infection is one of the serious problems that cause a great threat to the profitability of birds enterprises all over the world. Although *E. coli* is a normal inhabitant of the intestinal tract of birds, under the influence of predisposing factors, like inadequate and faulty ventilation, over crowding, hunger, thirst, extremes of temperatures become pathogenic and lead to air sacculitis, pericarditis, perihepatitis and peritonitis, also high mortality during rearing, reduced weight gain and condemnation of birds at the time of slaughter (Ewers *et al.*, 2003). The genus *Salmonellae* comprise over 2700 serotypes that are found in different hosts and environment. It can cause human illness, including enteric fever, gastroenteritis and septicemia. *Salmonella* is one of the major bacterial agents that cause food borne infections in humans worldwide (Herikstad

*et al.*, 2002). Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with avian colibacillosis. However, resistance to existing antimicrobials is widespread and of concern to poultry veterinarians (Blanco *et al.*, 1998). Antimicrobial resistance originated from change in the microbial metabolism and their genetic structure .Within the last few decades, microbial resistance has emerged for most of the available agents, thus necessitating the search for newer drugs. Medicinal plant oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties. Bhattacharjee *et al.*, 2005 and Raghunath, 2008.). Most the active principles of oils consists of mixtures of compounds such as phenolics and polyphenols, terpenoides, saponins, quinines, esters, flavones, flavonoids,

tannins, alkaloids and nonvolatile residues. Their chemical composition and concentration of compounds is variable. These components have many effects as antimicrobial, stimulating animal digestive systems, antioxidants, anticoccidial and increase production of digestive enzymes, also it improve utilization of digestive products by enhancing liver functions (Hernandez *et al.*, 2009). Therefore, the present work was planned to determine the antibacterial effect of some medicinal plant oils against *E. coli* and *salmonellae* in vivo.

## 2. MATERIAL AND METHODS

### 2.1. Samples collection:

A total of 100 samples from Al-Gharbia Government were collected, (75 samples from diseased chickens suffered from diarrhea and respiratory manifestation and 25 samples from recently dead ). All samples (liver, lungs, intestine, spleen and heart) were aseptically collected and transferred immediately in icebox to the laboratory.

### 2.2. Bacteriological examination of the samples

The samples were identified on the basis of Gram staining, after streaking onto media as MacConky's agar, EMB, XLD and S.S agar media. Each colony showed typical colonial appearance were subjected to biochemical identification and examined for indole, MR and Simmons citrate tests. The cultural characterization and biochemical screening-routine methods were used according to (Quinn *et al.*, 2002).

### 2.3. Serological identification

#### 2.3.1. Diagnostic *E. coli* antisera

The isolates were serogrouped using Sifin antisera which involved four vials of polyvalent and 24 vials of monovalent diagnostic *E. coli* antisera according to somatic (O) antigen.

#### 2.3.2. Diagnostic *Salmonella* Antisera

Diagnostic polyvalent, monovalent I, II, III and monovalent *Salmonella* O and H (phase 1 and phase 2) antisera (Denka Seiken co., LTD) & (Pro – lab diagnostic, U.K).

### 2.4. Detection of MIC and MBC of medicinal plant oils

(CLSI., 2009) by micro titre plates. Cation adjustment Muller Hinton broth, DMSO 5%, Essential oil and Resazurin dye were used. Five medicinal plant oils were used in the present study: Garlic, Thyme, Mint , Cinnamon and Eucalyptus oils are obtained from unit of essential oils in National Research Center.

## 3. RESULTS

A total of 100 samples were collected (75 samples from diseased broiler chickens and 25 samples from recently dead broiler chickens) from Tanta, Kotour, Basione and Kafer-Elzyat. Clinically examined birds showed signs of listlessness, ruffled feathers, labored breathing and coughing. P.M lesions were enteritis, septicemia, airsacculitis, perihepatitis, pericarditis, peritonitis, synovitis and omphalitis. The samples were examined bacteriologically which revealed Fifty two isolates of *E. coli* (52%) and seven isolates of *Salmonella* (7%) were detected, (Table1 ). Ten random samples of *E. coli* were serogrouped and revealed that five were *E. coli* O78, two O157 and three untyped and seven isolates of *Salmonella* were serotyped and revealed that three *S. Enteritidis*, one *S. Charity* , one *S. Remiremont* and two untyped .

MIC and MBC of each medicinal plant oils were showed in that Eucalyptus oil found to be effective against *E. coli* O78 and O157 by MIC 80µg /ml and 1280µg /ml respectively, and MBC at 160 µg | ml and 2560µg | ml respectively. Eucalyptus oil found to be effective against *S. Enteritidis*, *S. Charity* and *S. Remiremont* by MIC 20µg /ml, 5120µg /ml and 2560µg /ml respectively and MBC at 40µg |ml for *S. Enteritidis* and 5120 µg| ml for *S. Remiremont* but did not have MBC for

*S. Charity* (table 2). Mint oil found to be effective against *E. coli* O78 and O157 by MIC 320 µg/ml and 2560µg /ml respectively and MBC at 320µg/ml and 2560 µg/ml respectively. and also effective against *S. Enteritidis* ,*S. Charity* and *S. Remiremont* by MIC 5µg /ml, 5120µg /ml and 2560 µg/ml respectively and MBC at 5µg |ml for *S. Enteritidis* and 2560µg |ml for *S. Remiremont* but did not have MBC for *S. Charity* (table 2). Cinamoon oil found to be effective against *E. coli* O78 and O157 by MIC 2560µg /ml and 5120µg /ml respectively and MBC at 2560 µg/ml for *E. coli* O78 only but did not have MBC for O157 and also effective against *S. Entertidis* and *S. Remiremont* by MIC 5µg /ml and 5120 µg/ml and MBC at 10µg |ml for *S. Entertidis* but not effective against

*S. Charity* and did not have MBC for *S. Remiremont* (table 2). Garlic oil found to be effective against *E. coli* O78 and O157 by MIC 320µg /ml 1280 µg/ml respectively and MBC at 1280µg \ml and 2560 µg/ml respectively and also effective against *S. Entertidis*, *S. Charity* and *S. Remiremont* by MIC 5µg/ml ,5120µg /ml and 160µg /ml respectively and MBC at 20µg |ml for *S. Entertidis* and 320µg/ml for *S. Remiremont* but did not have MBC for *S. Chairty* (table 2). Thymus oil found to be effective against *E. coli* O78 and O157 by MIC 2560 µg /ml and 5µg /ml respectively and MBC at 2560µg |ml and 10 µg /ml respectively and also effective against *S. Entertidis* and *S. Remiremont* by MIC 320µg /ml and by MBC at 320 µg /ml but not effective against *S. Charity* (table, 2)

Table (1): Prevalence of *E. coli* and *Salmonella* species infection in chickens

	No. of collected samples	No. of positive samples			
		E.coli		Salmonella	
		No.	%	No.	%
Diseased birds	75	48	64	5	6.6
Recently dead birds	25	4	16	2	8.0
Total	100	52	52	7	7.0

Table (2): Minimum inhibitory concentrations (MICS) and Minimum bactericidal concentrations (MBCS) results of medicinal plant oils against *E. coli* and *Salmonella* species.

oils	Eucalyptus		Mint		Cinnamon		Garlic		Thyme	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Strains	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml
<i>E. coli</i> O78	80	160	320	320	2560	2560	320	1280	2560	2560
<i>E. coli</i> O157	1280	2560	2560	2560	5120	-	1280	2560	5	10
<i>S. Entertidis</i>	20	40	5	5	5	10	5	20	320	320
<i>S. Chairty</i>	5120	-	5120	-	-	-	5120	-	-	-
<i>S. Remiremont</i>	2560	5120	2560	2560	5120	-	160	320	320	320

#### 4. DISCUSSION

Medicinal Plant oils and extracts have been used in food preservation, pharmaceuticals, alternative medicine and natural therapies (Reynolds, (1996) and Lis-Balchin and Deans (1997). In the present study, 52 isolates of *E.coli* were isolated from 100 samples (75 samples from diseased broiler chickens and 25 samples from recently dead). This result was nearly similar to that recorded by Akond *et al.*, (2009) and Wafaa, (2012). In addition, seven isolates of *Salmonella* (7%) were isolated. The obtained result was nearly similar to that recorded by Abd-Allah (1991). In the current study ten random samples of *E.coli* were serogrouped five were *E. coli* O78, two O157 and three untyped, the results agreed to Taha (2002) and Marwah *et al.*, (2010). Concerning to serotyping for seven isolates of *Salmonella* revealed that three *S.Enteritidis*, one *S.Charity*, one *S.Remiremont* and two untyped. These results supported by Marin and Lainez (2009) who recorded that *S.Enteritidis* was the most prevalent serotype isolated from broiler. Eucalyptus oil found to be effective against *E.coli* O78 and O157 by MIC 80 µg/ml and 1280 µg/ml respectively and MBC at 160 µg/ml and 2560 µg/ml respectively. Also effective against *S.Enteritidis*, *S.Charity* and *S.Remiremont* by MIC 20 µg/ml, 5120 µg/ml and 2560 µg/ml respectively. MBC at 40 µg/ml for *S.Enteritidis* and 5120 µg/ml for *S.Remiremont* but did not have MBC for *S.Charity*. These results agreed with Ayepola and Adeniyi, (2008).

Mint oil found to be effective against *E. coli* O78 and O157 by MIC 320 µg/ml and 2560 µg/ml respectively and MBC at 320 µg/ml and 2560 µg/ml respectively and also effective against *S. Enteritidis*, *S. Charity* and *S. Remiremont* by MIC 5 µg/ml, 5120 µg/ml and 2560 µg/ml respectively. MBC at 5 µg/ml for *S. Enteritidis* 2560 µg/ml for *S. Remiremont* but did not have MBC for *S. Charity*. The results were in agreement with Awang (1998), Clark,

(2002) and Iscan *et al.*, (2002) who mentioned that Menthol is known as a disinfectant with effective antimicrobial properties, which is the main component of Mint oil. Garlic oil found to be effective against *E.coli* O78 and O157 by MIC 320 µg/ml 1280 µg/ml respectively; and MBC at 1280 µg/ml and 2560 µg/ml respectively. And also effective against *S.Enteritidis*, *S.Charity* and *S.Remiremont* by MIC 5 µg/ml, 5120 µg/ml and 160 µg/ml respectively and MBC at 20 µg/ml for *S.Enteritidis* and 320 µg/ml for *S.Remiremont*. However, did not have MBC for *S.Charity*. The above results were agreed with reported by Hannan *et al.*, (2011). Cinnamon oil found to be effective against *E.coli* O78 and O157 by MIC 2560 µg/ml and 5120 µg/ml respectively and MBC at 2560 µg/ml for *E.coli* O78 only but did not have MBC for *E.coli* O157. Also effective against *S.Enteritidis* and *S.Remiremont* by MIC 5 µg/ml and 5120 µg/ml and MBC at 10 µg/ml for *S.Enteritidis*. However, not effective against *S. Charity* and did not have MBC for *S.Remiremont*. These results were in harmony to that reported by Bullerman *et al.*, (1977), Simic *et al.*, (2004) and Seenivasan *et al.*, (2006) who mention that the major antimicrobial components of spices and their essential oils are cinnamic aldehyde and eugenol in Cinnamon, Cinnamaldehyde was the predominant active compound found in cinnamon oil. Thyme oil proved to be effective against *E.coli* O78 and O157 by MIC 2560 µg/ml and 5 µg/ml respectively and MBC at 2560 µg/ml and 10 µg/ml respectively and effective against *S. Enteritidis* and *S. Remiremont* by MIC 320 µg/ml and by MBC 320 µg/ml but not effective against *S. Charity*. The result in parallel to that observed by Irena *et al.*, (2009) who found that thyme had the highest antibacterial efficiency against tested food borne bacteria strains *S. Enteritidis* and *E. coli*. It could be concluded that Eucalyptus and Mint oils had antibacterial activity against *E. coli* and *Salmonella* species at lowest MIC in vivo

and advice to evaluate the physiological effects of these oils in vivo.

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