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Evaluating the Efficacy of Some Antibiotics and Medicinal Plant Extracts Against the Infection of *Escherichia Coli* and *Salmonella Enteritidis* and Their Effect on Poultry Meat Quality

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Abstract

The present study was conducted to evaluate the efficacy of some antibiotics and methanol extracts of both *Punica granaturm* (pomegranate) peel and *Cymbopogon citrates* (lemon grass) against *Escherichia coli* and *Salmonella Enteritidis* (SE) and comparing between them in vitro using sensitivity test and minimum inhibitory concentration (MIC) and in vivo model with regarding to their interactions in broiler chickens, evaluating the antioxidant activities of pomegranate and its effect on poultry meat quality. According to the sensitivity tests, levofloxacin (Levo) and pomegranate peel extract (PPE) were found to have the most effective antibacterial effect against SE. The minimum bactericidal concentration (MBC) of PPE was 46.8 mg/ml and MIC ranged from 1.4 to 23.4 mg/ml. One hundred and twenty, one day old broiler chicks, were divided into 8 equal groups: G1:negative control group without infection, G2: positive control group orally challenged with SE ($3x\ 10^8$) CFU/ml at the 10^{th} day of age, G3: infected group treated with levo (10 mg/kg) and PPE (125 mg/ml), G4: infected group treated with levo (10 mg/kg) and PPE (250 mg/ ml), G5: prophylactic group treated with PPE (500 mg/ ml) before infection, G6: infected group treated with levo (10 mg/kg), G7: infected group treated with PPE (125 mg/ ml) and G8: infected group treated with PPE (250 mg/ ml) via crop gavage. Blood and meat quality tests were performed after treatment and at the end of the study at 42 days. Biochemical parameters were carried out included detection of endogenous antioxidants (Malondialdehyde-Thiobarbituric Acid (MDA-TBA) and superoxide dismutase (SOD)), total serum protein, albumin, globulin and albumin/ globulin ratio. Quality tests included the organolyptic examination, PH, total volatile nitrogen (TVN), tissue TBA and microbiological tests. The obtained data showed that both doses of PPE exert similar bactericidal activity as the standard antibiotic (levofloxacin) with antioxidant effect and significantly ($P<0.05$) improved both blood parameters and meat quality. Both groups G3 and G5 still have infection. The biochemical analysis revealed that no significant change was recorded among groups in total serum protein, albumin, globulin or A/G ratio after treatment or at the end of the experiment. After treatment, G4 showed a remarkable reduction in blood

MDA levels when compared with normal or infected control groups. Regarding to SOD levels after treatment G2 and G5 showed a remarkable decrease while G3, G4, G6, G7 and G8 showed a significant increase. There was no significant difference was recorded at the end of the experiment between the tested groups. The overall results of this study suggested beneficial use of PPE as natural alternative therapeutic agent to synthetic antibiotics for treatment of *S. Enteritidis* infection in broilers' but not as a prophylactic. PPE give us a great chance in combating microbes and improving meat quality and safely either bacteriologically or chemically.

Key words: Pomegranate peels extract (PPE), antibacterial activity, minimum inhibitory concentration, antioxidant, *S. Enteritidis*, broilers, meat quality.

Introduction

Salmonella is a facultative gram-negative intracellular bacteria infecting wide range of hosts. It is one of the most important infectious poultry diseases, causing a high death rate and economical losses **(Ogunleye *et al*., 2009)**. *S. Enteritidis* is one of the most salmonella serotype in poultry products that associated with human salmonellosis **(Haiqi *et al*., 2013)** and considered as an important international public health and economic problem resulting in syndromes such as enteric fever, bacteremia, focal infection and enterocolitis. Therefore human health protection by the elimination of foodborne pathogens from food animals and their products has become very important for all sectors of the food production chain **(Thirabunyanon and Thongwittaya, 2012)**.

Fluoroquinolones and tetracyclines are the antibiotics most commonly used to treat Salmonella and until recently most strains were susceptible to these drugs. However, a high incidence of Salmonella resistant strains to commonly prescribed antibiotics have recently been reported in many countries further exacerbates this problem **(Choi *et al*., 2005)**. In addition to inducing resistance, antibiotics are sometimes produces adverse toxicity to the host organs, tissues and cells besides associated with opposing effects such as hypersensitivity, immune-suppression and allergic reactions **(Ahmad *et al*., 1998)**. Levofloxacin is a synthetic bactericidal antibiotic belonging to the fluoroquinolone that are used to control pulmonary, urinary and digestive bacterial infections in poultry and animals.

Nature has been a source of medicinal agents for thousands of years and since the beginning of man. It is important to find alternate antibiotics which are not only safer but also efficient **(Buchanan *et al*., 2008)**. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with side effects **(Deepa *et al*., 2012)**. The use of organic acids, probiotics and plant extracts in poultry diets has been reported as successful substitutes for antibiotics with positive effects on poultry

(Khosravi *et al*., 2008). Lemon grass (*Cymbopogon citrates)* was used in traditional medicine. It belongs to the Poaceae family, and a native to warm temperate and tropical regions **(Ernst, 2008)**. In recent years, the number of scientific papers concerning pomegranate (*Punica granatum* L.) and its health properties has increased greatly. It has gained widespread popularity as a functional food and nutraceutical source focused on the benefits of naturally occurring phytochemicals that exhibit potent antioxidant effects as potential antibacterial agents **(Mansy *et al*., 2015)**. Pomegranate is a native Mediterranean traditional plant belonging to family punicaceae that has been widely used by ancient Egyptians in the folklore medicine for the treatment of various diseases **(Tayel and El-Trase, 2010)**. The peels of pomegranate represent a valuable waste of the food industry as they contain bioactive compounds especially, polyphenols which are extracted from plant materials by organic solvents **(Cam and Hisil, 2010)**. Pomegranate fruit extract is a rich source of two types of polyphenolic compounds: anthocyanins (such as delphinidin, pelargonidin and cyanidin), which give the fruit and juice its red color, and the hydrolysable tannins (such as punicalin, pedunculagin, punicalagin, gallagic and ellagic acid esters of glucose), which account for 92% of the antioxidant activity of the whole fruit **(Afaq *et al*., 2005)**.

World Organization for Animal Health, (2010) clarified that although a variety of methods exist, the goal of *in-vitro* antimicrobial susceptibility testing is the same: to provide a reliable predictor of how a microorganism is likely to respond to antimicrobial therapy in the infected host in vivo and guide antibiotic choice.

Therefore, the present study was carried out to investigate and compare the antibacterial efficacy of methanol extracts of both lemon grass and pomegranate's peel and some antibiotics in controlling the pathogenic enteric microorganisms (*E.coli* and *S. Enteritidis)* in vitro using disc diffusion and minimum inhibitory concentration (MIC) tests and found out the highest antimicrobial activity of them to use in vivo model regarding to their interactions in broiler chickens by monitoring their effect on some blood biochemical parameters and poultry meat quality.

Material and Methods

Preparation of plant extracts

The extracts were prepared and obtained from the National research center, Egypt. Methanolic pomegranate peel extract (PPE) was done according to **Ramadan *et al*., (2015)** and methanolic lemon grass extract was extracted using the standard methods of **Ewansiha *et al*., (2012)**. Both extracts were filtrated, concentrated using a rotary evaporator to evaporate the solvent, lyophilized to obtain powders then converted to be water soluble substance. The extracts were stored in clean air tight containers at the refrigerator 4 °C until needed for use.

Tested bacterial strains

The two bacterial strains *E.coli* O157:H7 and *S. Enteritidis* (ATCC 13076) were obtained from biotechnology department, Animal health research institute, Giza, Egypt.

Standardization of inoculums

Four to five colonies from pure culture of each test organisms were transferred from an agar medium to 5 ml of Molar Hinton broth and incubated overnight at 35-37°C for 18-24 h. The bacterial inoculums were adjusted photometrically using a spectrophotometer at 625nm to give absorbance from (0.08-0.1) to be approximately (1.5×10^8) CFU/ml equal to 0.5 McFarland's standard for in vitro determination of antibacterial activity of the tested extracts (agar disc diffusion and MIC assays) according to **EUCAST, (2003)**.

In vitro antibacterial activity screening tests

Sensitivity test

The agar paper disc diffusion method was used according to **Jorgensen *et al*., (1999)**. Different commercially available standard antibiotic discs (Oxoid Ltd. Basingstoke Hampshine England) were used including levofloxacin (LEV 5µ/ml), cefoperazone (CEP 75µ/ml), oxytetracyclin (OT 30µ/ml), norfloxacin (NOR 10µ/ml), chloramphenicol (C 30µ/ml), Trisol (Ampicilin/colistin) in accordance with **Clinical and Laboratory Standards Institute, (2001)** guidelines, while sterilized paper discs (6mm) were soaked in known 4 different concentrations (0.25, 0.5, 1 and 1.5 gm/ml) of both extracts against the infection of *E. coli* and *S. Enteritidis.* Sterilized paper discs with sterilized distilled water, the solvent, were used as a negative control. Each experiment was conducted simultaneously in triplicate for both plant extracts and antibiotics. After incubation the formed growth inhibition zones were measured and mean values were calculated.

Minimum inhibitory concentration (MIC) assay

MIC was determined for PPE, the most active extract revealed by the previous screening disc diffusion test, against *S. Enteritidis*. A solution of 1.5 gm/ml concentration was two fold serially diluted to give extract concentrations of 750, 375, 187.5, 93.7, 46.8, 23.4, 11.7, 5.8, 2.9, 1.4 mg/ml. Micro-broth dilution test was performed in a 96 well microplate using standard procedure as described by **EUCAST, (2003)**. Dimethyl sulfoxide (DMSO, Sigma, USA) was used to indicate the presence of uninhibited bacterial growth in each well. Referring to the results of the MIC assay clear wells of the microtiter plate were sub-cultured for 24 h on the specific media for *S. Enteritidis*, XLD (oxoid England), and then observed for growth. The lowest concentration (highest dilution) that showed no turbidity (growth)

was recorded as the MIC and the lowest concentration did not show any growth was recorded as the minimum bactericidal concentration (MBC).

In vivo determination of antibacterial activity and evaluation of the synergistic effect of the used antibiotic and plant extract

Broilers' experiment

A total of 120 one day old broiler chicks were purchased from a local hatchery (Tanta city-Egypt) and were divided into 8 equal groups/15 birds each: G1:negative control group without infection, G2: positive control group orally challenged with *S. Enteritidis* (3x 10^8) CFU/ml, G3: infected group treated with levofloxacin (10 mg/kg) and PPE (125 mg/ml) and G4: infected group treated with levofloxacin (10 mg/kg) and PPE (250 mg/ml), G5: prophylactic group treated with PPE (500 mg/bird) before infection from 5^{th} to 9^{th} day of age, G6: infected group treated with levofloxacin (10 mg/kg), G7: infected group treated with PPE (125 mg/ml) and G8: infected group treated with PPE (250 mg/ml). All groups had the same management and vaccination with free access to commercial ration and water *ad libitum*. The oral infection was occurred at the 10^{th} day of age and treatment with PPE and/or levofloxacin were given after the appearance of the clinical signs and lasted for 5 successive days. Ten fold of the MIC dose of PPE 25 mg/ml and half this dose 12.5 mg/ml were given via crop gavage according to **Chuachan *et al*., (2006)**. The commercial recommended dose of levofloxacin Alveolin-S ® solution (10 mg/kg) was used via drinking water.

Blood samples and biochemical parameters

Blood samples were collected with anticoagulant from the wing vein for detection of plasma antioxidants using commercial colorimetric assay kits for Malondialdehyde-Thiobarbituric Acid (MDA-TBA) and superoxide dismutase (SOD) **(Yagi, 1984)**, while serum samples without anticoagulant were collected for detection of total protein **(Gornall, 1946)**, albumen **(Doumas *et al*., 1971)** with semi-automatic spectrophotometer (BM-Germany,5010) using commercial test kits (Randox Co. UK and Biodiagnostic, Egypt.). Globulin ratio was detected by subtraction albumin ratio from total protein and albumin/globulin ratio was detected by divided both ratios. All samples were collected after treatment and at the end of the experiment at 42 days.

Meat quality tests

Physical examinations: Samples were examined for color by naked eye, odor by roasting test using one gram of sample on direct flame and smelling the odor. The texture was also tested by touch and pressure.

Preparation of meat samples for bacteriological examination **(APHA, 1992)**, Aerobic Plate Count **(FDA, 2001)**, determination of total Staphylococci count **(APHA, 2001)**, determination of total Enterobacteriaceae count **(Anonymous, 1991)**,

Salmonella spp count **(Edao and Geue, 1998)**, chemical examination: determination of PH **(AOAC, 2000)** and determination of Total Volatile Nitrogen (TVN) and Thiobarbituric acid (TBA) **(Vynck, 1970)**. All tests were done in triplicate after treatment and at the end of the study.

Statistical analysis

Experimental data were assessed by one-way analysis of variance (ANOVA) - Duncan test using SPSS software statistical program (windows version 20.0, USA) to observe mean differences. Data were expressed as mean ± SE when (N=5) and were regarded as significant when ($P \leq 0.05$).

Results and Discussion

In vitro antibacterial activity screening tests

It is necessary to investigate those plants which have been used in traditional medicine scientifically to improve the quality of health care. According to the sensitivity test results in table (1), methanol extract of lemon grass extract had no antibacterial effect neither on *E. coli* nor *S. Enteritidis* while PPE gave a significant antimicrobial activity against the tested microorganisms. Its inhibition zone diameter (IZD) against *E. coli* and *S. Enteritidis* ranged from (15 to 25 mm). Results indicated superior antimicrobial activity of PPE against enteric bacterial infections than lemon grass extract even at the lowest concentration. The used antibiotic disks were chosen to reflect the range of drugs commonly prescribed for treatment of these infections. Levofloxacin showed the best IZD against both infections (23 mm for *E. coli* and 32 mm for *S. Enteritidis*). Both PPE and levofloxacin provided an approximate effectiveness on *S. Enteritidis*. The MIC is a well-established laboratory parameter routinely determined in microbiology. It is currently by far the most commonly used pharmacodynamic parameter for the evaluation of efficacy of anti-infective agents **(Mueller *et al*., 2004)**. The minimum bactericidal concentration (MBC) of PPE was found to be 46.8 mg/ml and MIC was ranged from 1.4 to 23.4 mg/ml. This extract exhibited both bacteriostatic and bactericidal activities indicating that it may be an effective adjunct treatment for *S. Enteritidis* infection. To some extent, our results were similar to those of previous studies which were focused on antimicrobial activity of pomegranate extracts. **Burt, (2004)** stated that gram-negative bacteria are more susceptible to the antibacterial activity of plant extracts. These compounds have hydrophobic characteristics and interact with different sites of microbial cell (e.g., cell wall and cytoplasmic membrane) causing loss of cellular constituents, collapse of membrane structure and cell death. Moreover, several studies as **Kannaiyan *et al*., (2013)** reported that ethanolic extract of pomegranate, showed a significant bactericidal activity against *S. enterica* (21.2 mm), *S. paratyphi* A (18.8 mm), *S. typhimurium* (18.6 mm) and *E. coli* (18.4 mm) with a MIC equal 1.024 μg/ml against

S. paratyphi A, *S. typhimurium* and *S. brunei*. The obtained results corresponds to the findings of **Chebaibi and Filali, (2013)** examined the antibacterial activity of PPE by sensitivity test against some pathogenic strains of *E. coli* and found that PPE had more or equally effective bactericidal activity as standard antibiotics with IZD ranged from 12.3 to 30.3 mm. **Jang-Gi Choi *et al.*, (2011)** recorded a significant antibacterial activity to PPE on *S. Enteritidis* ranged from 9.3 ± 1.1, 12.6 ± 0.5 and 14.6 ± 0.5mm corresponding to these concentrations of PPE (100, 200 and 500 µg/ml), while MIC ranged from (250-1000 µg/ml). Also **Duman *et al.*, (2009)** reported in vitro that antibacterial activity of alcohol extracts (methanol, ethanol, acetone) and water extracts of pomegranate were active and effective against *E. coli, S. typhi* and other tested M.O with IZD of 12-31 mm. Other studies have also reported the antibacterial activity of methanol extracts of pomegranate against foodborne pathogens including *E. coli* and Salmonella **(Dey *et al.*, 2012 and Ramadan *et al.*, 2015)**. **Altuner (2011)** stated that water extract of *P. granatum* fruit rind showed antibacterial activity against *E. coli* and *S. typhi*, and that ethanol and solvent cocktail extract of its peel induces antibacterial activity against *E. coli* and *S. enterica*. These differences in the antibacterial activity of PPEs among studies could be partially explained by variations in extraction methods, freshness of fruits, variations in the season and region of growth, strains sensitivity and antimicrobial procedures adopted in tests (**Opara *et al.*, 2009 and Al-Zoreky, 2009)**. Several authors **Reddy *et al.*, (2007), Al-Zoreky, (2009), Dahham *et al.*, (2010) and Hamady *et al.*, (2015)** have linked the antimicrobial properties of methanolic PPE to the presence of several phytochemical bioactive compounds such as phenolics, flavonoids, hydrolyzable water soluble tannins (punicalins and punicalagins), ellagic acid, a component of ellagitannins, and gallic acid, a component of gallotannins, terpenoids, phytosterols, glycosides and saponins. Also high content of total flavonols, phenolics, anthocyanins and organic acids was found by **Duman *et al.*, (2009)**. According to **Kanatt *et al.*, (2010)** phenolics is well known to possess antimicrobial therapeutic properties, it has the ability to disrupt the bacterial cell membrane, while **Opara *et al.* (2009)** associated this activity to the presence of vitamin C in pomegranate peel. Meanwhile, our lemon grass results are in contrary to the findings of **Lidiane *et al.*, (2009)** who investigated in vitro the antimicrobial activity of lemon grass against gram-negative strains (*E. coli* and *S. enteritidies*) and found a variable reduction values. Also our findings disagree with **Fagbemi *et al.*, (2009) and Adegbegi *et al.*, (2012)** who concluded that *S. paratyphi* and *E. coli* were susceptible to ethanolic extracts of lemon grass. However, our results were in accordance with that found by **Nkambule, (2008)** that no antimicrobial activity was

observed in any lemon grass leaf or stems extracted with various solvents on *E. coli* O157:H7 or *Salmonella* serotypes Enteritidis.

In vivo determination of antibacterial activity and evaluation of the synergistic effect of the used antibiotic and plant extract

The success of antimicrobial therapy is determined by complex interactions between an administered drug, a host and an infecting agent. In a clinical situation, the complexity of these interactions is usually reflected by a high variability in the dose-response relationship. To date, dose and drug selection is mostly based on a static in vitro parameter, the MIC and on the drug′s serum concentration as a pharmacokinetic parameter. Thus, it do by no means reflect the in vivo scenario, where bacteria are not being exposed to constant but constantly changing antibiotic concentrations, so in practice, a pharmacodynamic effect in vivo is needed **(Mueller *et al*., 2004)**.

The biochemical analysis in table (2) revealed that no significant change was recorded in total serum protein (TP), albumin, globulin or A/G ratio between groups after treatment or at the end of the experiment. The obtained results were contrary to the findings of **Kokosharov, (2006)** who observed that the TP, A/G ratio (0.14), β- and γ_2 globulins – significantly reduced in liver and blood serum while α^2 globulins were increased during the course of experimental oral infection with *S. gallinarum* in 6-month old chickens compared to non-infected control birds developed a hypoalbuminaemia, hypoproteinaemia and dysproteinaemia. Meanwhile, our result agree with **Osman *et al*., (2011)** who found that there was no significant effects were observed on the serum TP, albumin and globulin contents between PPE treated group and normal control while disagree with the author in recording a significant increase in TP and albumin indicating hepatoprotective and curative effect in rats treated with a dose of 50 mg pomegranate peel ethanolic extract daily for 21 consecutive days after administration of carbon tetrachloride (CCl_4). **Fatma *et al*., (2013)** investigated an increase in TP and globulin level in the infected group while albumin and A/G ratio insignificantly changed comparing with normal control group at the 1st week post *S. Enteritidis* challenge.

Malondialdehyde (MDA), a stable metabolite of the free radical mediated lipid peroxidation (LP) cascade, was widely used as marker of LP **(Osman *et al*., 2011)**. It is likely that MDA can form complexes with other biological components such as protein, lipids, and nucleic acids which can contribute to an under estimation of endogenous LP **(Shulaev and Oliver, 2006)**. After treatment, plasma MDA levels in table (2) showed a significant difference between groups. The remarkable reduction in its blood level was noticed in G4 the infected group treated with

levofloxacin (10 mg/kg) and PPE at (250 mg/bird) when compared with normal or infected control groups indicates inhibiting LP. However, MDA levels were also increase in treated groups, this may explained by increase LP of hepatocytes as *S. enterica* serovar Enteritidis bacterial LPS (endotoxin) induces extensive damage to a variety of organs, including liver due to the increased production of reactive oxygen intermediates **(Benzer *et al*., 2009)**. At the end of the experiment there was no significant difference was recorded between groups. These results were confirmed by meat quality testes used for detection of TBA in thigh and breast muscles. The data of **Chidambara *et al*. (2002 b) and Ajaikumar *et al*. (2005)** also showed that tissue LP level decrease in the methanol *P. granatum* extract treated groups of animals as compared to the control group. Our results agree with **Osman *et al*., (2011)** who found that there was a significant decrease in MDA level in PPE treated group than carbon tetrachloride (CCl_4) treated group while disagree with the author in recording a non significant difference with the normal control.

Interest in the search for new natural antioxidant has grown dramatically over the past years because reactive oxygen species production and oxidative stress has been shown to be linked to a large number of illnesses **(Finkel and Holbrook, 2000)**. Phytochemicals such as polyphenols (including the phenolic acids and flavonoids which are concentrated in pomegranates) have demonstrated antioxidant properties and can inhibit inflammation and other deleterious processes involved in degenerative diseases **(Aggarwal and Shishodia, 2004)**, improve digestion and metabolism besides have antibacterial and immune stimulant activities in animals **(Rajaian *et al*., 2013)**.

Superoxide dismutase (SOD) is the first enzyme involved in the antioxidant defenses against reactive oxygen species (ROS) by dismutation of superoxide anion ($O2^-$) to hydrogen peroxide (H2O2) **(Osman *et al*., 2011)**. After treatment plasma SOD levels in table (2) showed significant differences between groups. The remarkable decrease were recorded in G2: infected non treated group and G5: prophylactic group treated with PPE at (500 mg/bird) before infection indicating a bad prognosis of these groups when compared with normal control or other treated groups. Our results are in accordance with that found by **Fatma *et al*., (2013)** who recorded that SOD activity decrease either at 1^{st} or 2^{nd} week post *S. Enteritidis* infection. As known the cell has protective agents against damage induced by oxygen-reactive species (ROS) including GSH-Px, CAT and SOD that are constitute an antioxidant cellular enzymatic system. LPS-induced increase in ROS resulted in increase lipid peroxidation and nitric oxide levels and decrease in the antioxidant activity in tissues **(Benzer *et al*., 2009)**. A significant increase in the blood SOD levels were noticed in G3, G4, G6, G7 and G8 infected groups treated with PPE

and/or levofloxacin. This clearly explained the reason for the antioxidant activity of PPE indicating a significant improvement in body's defenses against ROS the harmful free radicals and oxidative stress. **Jurenka, (2008) and Dey *et al*., (2012)** demonstrated that the PPEs have synergestic activity when used in conjunction with other antimicrobial agents, therefore offering therapeutic potential. Our results agree with **Osman *et al*., (2011)** who found that treated rats with ethanolic PPE significantly increase the activity of SOD enzyme when compared with CCl4-treated group during the hepatoprotective and curative periods. Similar trend of the treated rats with PPE was observed by **Chidambara *et al*. (2002 b)** who found that pretreated rats with methanolic PPE at 50 mg/kg body weight for 14 days followed by CCl4 treatment causes preservation of SOD enzyme activity. Also **Ajaikumar *et al*., (2005)** found that the in vivo antioxidant level of SOD in PPE treated groups of animals was increased near to the normal values. There was no significant difference was recorded at the end of the experiment between groups and this may explained by ending the effect of PPE on blood antioxidants by time.

Our data suggests that PPE can increase the endogenous antioxidant defense and decrease oxidative stress. An imbalance in the function of endogenous antioxidant defense mechanisms can lead to the accumulation of free radicals and oxygen-reactive species (ROS) and increased susceptibility to oxidative stress, which contributes to the pathogenesis of the diseases. These results provide promising baseline information for the potential use of this plant extract in the treatment of enteric (foodborne) diseases in poultry.

Meat and meat products are susceptible to quality deterioration as chemical and microbial changes due to their rich nutritional composition **(Devatkal *et al*., 2012)**. The most common form of chemical deterioration is the oxidation of meat lipids. Lipid oxidation is a complex process and depends on chemical composition of meat, light and oxygen access and storage temperature **(Kanner, 1994)**.

So, from the achieved results in this study we realized from table (3) that G1 and G3 had the best bacterial count in comparison with the other groups specially G2 and the reason could be due to the effect of the PPE however G1 was like as it was the control one and also the salmonella free one too, following good results given by the antibiotic with and/or PPE and this agreed with the findings of **Nannapaneni *et al*., (2008); Al-Zoreky, (2009) and Rakholiya *et al*., (2014)**.

After the finishing of the rearing period (experiment time with a marketing weight) we noticed that in table (4) there were a great changes in the microbial load in all groups and the best achievement given by G4 and G5 followed by G6, G7 and G8 and this could be attributed to the higher the concentrations of the PPE the power on killing of the microbes achieved and this also happened on the antibiotic

concentration and these results were co-in same with those reported by **Nannapaneni *et al*., (2008) and Hamdan *et al*., (2010)**.

Pomegranate extract polyphenols, especially tannins are the major components in the PPE extract that have been implicated in antimicrobial potential (antiviral, antifungal and antibacterial activities) **(Miguel *et al*., 2010)**. Phenolic compounds have been suggested to degrade cell wall protein, disrupt cytoplasmic membrane and interfere with membrane integrated enzymes **(Shan *et al*., 2007)**. It has also been suggested that the antimicrobial activity of tannins may be due to their ability to precipitate proteins, therefore causing leakage of cell membrane of the microorganism **(Endo *et al*., 2010)**, and aiding cell lysis and death meanwhile, **Kanatt *et al*., (2010)** found that pomegranate extracts showed little or no effect on Gram negative bacteria.

By talking about the sensory evaluation and pH of the tested groups there was a significant differences between them but all showed acceptability except G2 at the beginning and also at the end of the trial.

Thiobarbituric acid value in pomegranate treated groups in table (5) showed also an increased trend but it should be noted that pomegranate treated samples showed lower pH and TBA no when compared to non-treated meat samples. In this respect, TBA reactive substance values were lower in chicken meat pomegranate extract solution **(Vaithiyanathan *et al*. 2011)** and this clearly noticed in G4 and G8 while followed by the other groups (G5,G6 and G7) and this agreed with the findings of **(Ahmed *et al*., 2015)**. The effect of PPE in maintaining TBA at lower values could be attributed to the antioxidant effects which have been proved by other investigations **(Fawole *et al*., 2012 and Hasnaoui *et al*., 2014)**.

In the present study a lower TVN in pomegranate treated samples in table (5) was reported. The effect of PPE on TVN level could be attributed to its antioxidant and/or antibacterial activity, since this effect is clear in samples polluted with *S. entertidis*.

It has been reported that a combination of microbiological and autolytic action the antimicrobial activity of PPE has also been previously confirmed by **(McCarrell *et al*. 2008 and Endo *et al*. 2010)**. More recently, it has also been reported that PPE aqueous extract has an antibacterial activity against four bacterial strains, including *S.aureus*, and *S. typhimurium* **(Malviya *et al*. 2014)**.

The findings in this study are in agreement with published reports that found similar antibacterial activity of peel extracts **(Nannapaneni *et al*., 2008; Al-Zoreky, 2009; Tayel *et al*., 2012 and Rakholiya *et al*., 2014)** on selected bacteria. It has also been demonstrated that the PPE have synergistic activity when used in conjunction with other antimicrobials. However, reports also exist on the failure of some of these

extracts to inhibit certain bacteria and that different fractions of the extracts vary in their antimicrobial activities **(Nannapaneni *et al*., 2008 and Sah *et al*., 2011)**.

Conclusion:

Methanol extract of *P. granaturm* (pomegranate) peel had higher antibacterial activity against *E. coli* and *S. Enteritidis invitro* than *C. citrates* (lemon grass) extract.

PPE is one of the medicinal plants, posses a bactericidal activity as levofloxacin antibiotic against *S. Enteritidis* that makes it a good choice as an effective natural antibiotic, antioxidant, decreasing lipid peroxidation, oxidative stress and increasing the biological activities of the birds to overcome the infection, give us a great chance in combating microbes and improving meat quality safely and also in an easy way.

The source of this extract may increase their acceptance by ever-increasing health conscious consumers without any deleterious effect on health.

Table (1); Effect of different methanolic extracts and antibiotics on the inhibition zone diameter in mm (IZD) against both *E. coli* and *S. Enteritidis* growth (Mean ± SE).

Extracts' disc conc. (mg)	*P. granaturm*		*C. citrates*		Antibiotic discs	*E. coli*	*S. Enteritidis*
	E. coli	*S. Enteritidis*	*E. coli*	*S. Enteritidis*	Levofloxacine	23±0.06*	32 ± 1.00*
250	15 ± 0.01	15 ± 0.07	R	R	Cefoperazone	18 ± 0.9	30 ± 1.00*
500	15 ± 0.01	20 ± 0.01	R	R	Oxytetracycline	20 ± 0.01	25 ± 0.8
1000	17 ± 0.07	23 ± 0.01	R	R	Norfloxacine	20 ± 0.2	30 ± 1.00*
1500	25 ± 0.01*	25 ± 0.08*	R	R	Chloramphinicol	22 ± 0.4*	28 ± 0.8
					Trisol	17 ± 0.08	18 ± 0.7

*indicates statistically significant differences in the same colon when (P≤0.05). R means resistant.

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Table (2): Effect of PPE and/or levofloxacin treatment on serum biochemical parameters and antioxidants (Mean ± SE) after treatment and at the end of the experiment.

	Group	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	MDA (nmol/ml)	SOD (u/ml)
After treatment	1	6.23 ± 0.13 a	4.87 ± 0.04 b	1.36 ± 0.15 a	3.58 ± 0.10 a	10.64 ± 0.05 b	12.18 ± 2.12 c
	2	6.29 ± 0.09 a	4.90 ± 0.07 b	1.39 ± 0.08 a	3.53 ± 0.08 a	15.76 ± 0.24 a	4.28 ± 1.74 e
	3	6.33 ± 0.08 a	4.99 ± 0.05 a	1.34 ± 0.07 a	3.73 ± 0.06 a	9.47 ± 0.27 b	15.80 ± 1.56 b
	4	6.14 ± 0.18 a	4.90 ± 0.04 b	1.24 ± 0.22 a	3.95 ± 0.13 b	7.13 ± 1.67 c	18.80 ± 2.73 a
	5	6.26 ± 0.05 a	4.96 ± 0.04 a	1.30 ± 0.11 a	3.82 ± 0.07 b	13.67 ± 1.31 a	8.27 ± 1.56 d
	6	6.27 ± 0.09 a	4.93 ± 0.05 ab	1.34 ± 0.08 a	3.68 ± 0.06 a	15.33 ± 1.54 a	20.01 ± 0.85 a
	7	6.27 ± 0.05 a	4.95 ± 0.05 a	1.32 ± 0.09 a	3.75 ± 0.07 a	13.10 ± 1.23 a	20.70 ± 1.00 a
	8	6.25 ± 0.09 a	4.93 ± 0.06 ab	1.32 ± 0,13 a	3.73 ± 0.10 a	13.33 ± 1.87 a	18.67 ± 1.75 a
At the end of the experiment	1	6.00 ± 0.10a	5.07 ± 0.03a	0.94 ± 0.05a	5.39 ± 0.05a	10.30 ± 0.08a	12.07 ± 1.52a
	2	6.06 ± 0.05a	5.08 ± 0.05a	0.98 ± 0.04a	5.18 ± 0.03a	10.73 ± 0.08a	11.53 ± 1.17a
	3	6.45 ± 0.09a	5.10 ± 0.07b	1.35 ± 0.08b	3.78 ± 0.09b	9.98 ± 0.08a	11.87 ± 0.98a
	4	6.37 ± 0.08a	5.12 ± 0.04b	1.25 ± 0.12b	4.10 ± 0.09c	10.00 ± 0.08a	12.02 ± 1.33a
	5	6.24 ± 0.04a	5.02 ± 0.10a	1.22 ± 0.07b	4.11 ± 0.08c	10.70 ± 0.08a	11.89 ± 2.02a
	6	6.28 ± 0.10a	5.04 ± 0.03a	1.38 ± 0.03b	3.65 ± 0.05b	10.40 ± 0.08a	12.03 ± 1.72a
	7	6.22 ± 0.18a	5.10 ± 0.07b	1.23 ± 0.05b	4.15 ± 0.06c	10.10 ± 0.08a	12.09 ± 1.54a
	8	6.03 ± 0.18a	4.78 ± 0.12c	1.09 ± 0.23a	4.39 ± 0.10d	9.99 ± 0.08a	12.10 ± 1.09a

The various letters in the same colon indicate statistically significant differences when ($P < 0.05$).

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Table (3): Effect of PPE and/or levofloxacin treatment on meat quality tests (Mean ± SE) after treatment.

G	PH	T.B.C	*Salmonella Enteritidis*	*Staphylococcus aureus*	*Enterobactereacae*	*Total Psychotroph*
1	5.5±0.01 A	$30x10^5±1.2x10^4$ A	Free	$2x10^3±1.2x10^2$ A	$36x10^3±12x10^2$ A	$3x10^2±3.02x10^2$ A
2	6.5±0.3 B	$77x10^6±5.6x10^5$ C	$32x10^6±6.3x10^5$ C	$13x10^5±2.02x10^5$ C	$5.9x10^4±2.6x10^3$ A	$62x10^3±3.3x10^2$ B
3	5.5±0.02 A	$36x10^5±3.5x10^4$ A	$6x10^3±2.2x10^3$ B	$91x10^3±5x10^2$ A	$22x10^3±1x10^2$ B	$25x10^2±1.2x10^2$ A
4	5.5±0.01 A	$5x10^6±2.8x10^5$ C	Free	$23x10^4±2.2x10^4$ B	$16x10^3±2.2x10^2$ B	$36x10^3±2.6x10^2$ B
5	5.5±0.01 A	$65x10^5±2.5x10^5$ B	$42x10^2±0.2x10^2$ A	$63x10^3±3.2x10^3$ A	$2x10^3±5x10^2$ B	$95x10^3±5.2x10^2$ B
6	5.0±0.2 C	$33x10^6±5.1x10^5$ C	Free	$55x10^3±3.9x10^3$ A	$9x10^3±3.6x10^3$ B	$36x10^2±2.5x10^2$ A
7	5.5±0.02 B	$8x10^6±1.2x10^6$ C	Free	$36x10^4±1.1x10^3$ B	$16x10^3±12x10^3$ B	$53x10^3±6.1x10^3$ B
8	5.5±0.01 C	$15x10^5±3.2x10^4$ A	Free	$18x10^3±2.0x10^2$ A	$5x10^2±1.1x10$ A	$22x10^2±23.0x10^2$ A

The various letters in the same colon indicate statistically significant differences when ($P < 0.05$).

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Table (4): Effect of PPE and/or levofloxacin treatment on meat quality tests (Mean ± SE) at the end of the experiment.

G	PH	T.B.C	*Salmonella Enteritidis*	*Staphylococcus aureus*	*Enterobacterieacae*	*Total Psychotroph*
1	5.5±0.01 A	$55x10^6±25x10^5$ C	Free	$20x10^4±5.0x10^4$ C	$36x10^4±1.2x10^4$ B	$3x10^3±5x10^2$ A
2	6.5±0.3 B	$76x10^6±3.1x10^5$ C	$25x10^6±1.2x10^5$ A	$13x10^3±2.0x10^4$ B	$37x10^5±8.4x10^4$ C	$66x10^3±21x10^2$ B
3	6±0.02 B	$30x10^6±2.2x10^6$ B	$41x10^3±2x10^3$ B	$25x10^4±2.1x10^3$ B	$63x10^6±1.8x10^5$ D	$53x10^3±1.5x10^2$ C
4	5.5±0.01 A	$31x10^6±1.6x10^5$ B	Free	$11 x10^4±2.0x10^3$ B	Free	$2x10^3±1.1x10^2$ A
5	5.5±0.01 A	$3x10^6±8.0x10^5$ A	Free	$8x10^4±1.3x10^3$ B	Free	$37x10^3±5.7x10^2$ B
6	5. 5±0.1 A	$2x10^6±5.1x10^5$ C	Free	$50x10^4±20x10^4$ C	$55x10^5±2.3x10^5$ C	$52x10^3±1.4x10^2$ C
7	5.5±0.01 AB	$8x10^6±5x10^5$ A	Free	$36x10^4±2.2x10^3$ D	Free	$3x10^4±8.1x10^3$ C
8	5.5±0.01 A	$15x10^3±3.3x10^3$ C	Free	$19x10^3±5.1x10^2$ A	$20x10^3±2x10^2$ A	$64x10^3±2.2x10^2$ B

The various letters in the same colon indicate statistically significant differences when ($P < 0.05$).

Table (5): Effect of PPE and/or levofloxacin treatment on TVN and TBA meat quality (Mean ± SE) after treatment and at the end of the experiment.

G	TVN		TBA	
	After treatment	At the end of the experiment	After treatment	At the end of the experiment
1	18.06±0.24 ^{C}	10.75±0.92^{A}	0.33±0.03 ^{C}	0.17±0.02^{D}
2	34.94±0.36 ^{D}	21.50±0.08 ^{D}	2.13±0.1^{DA}	1.02±0.01^{A}
3	15.81±0.05 ^{B}	13.44±0.082 ^{B}	0.11±0.05 ^{A}	0.38±0.06 ^{C}
4	5.68±0.62 ^{A}	10.27±0.02 ^{A}	0.14±0. 2 ^{A}	0.09±0.02 ^{A}
5	16.81±0.01 ^{B}	18.82±0.01 ^{C}	0.13±0.01 ^{A}	0.24±0.03 ^{B}
6	17.3±0.25^{B}	16.13±0.51^{B}	0.17±0.02 ^{A}	0.09±0.03^{A}
7	15.6±0.013 ^{B}	12.34±0.32^{B}	0.16±0.02 ^{A}	0.32±0.02^{C}
8	6.24±0.51 ^{A}	10.94±0.26 ^{A}	0.08±0.51 ^{A}	0.26±0.01^{B}

The various letters in the same colon indicate statistically significant differences when (P <0.05).

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