**Synergistic antimicrobial combination with cumin extract and some antibiotics on *Staphylococcus aureus* from chickens**

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**Abstract**

**In the present study antimicrobial activity of methanol Cuminum cyminum extract (50mg/ml) in combination with some antibiotics were investigated against *Staphylococcus aureus* isolates.** **The antimicrobial sensitivity pattern of these isolates in vitro was done and the intermediate isolates were checked for combination with methanol Cuminum cyminum extract after detecting their antimicrobial activities against these isolates by both disc diffusion test and MIC and the results showed that the antimicrobial activities of rifampicin, enrofloxacin, doxycycline, gentamicin, and erythromycin were enhanced in combination with methanol Cuminum cyminum extract the obtained data showed that enhancement by both disc diffusion test and MIC for methanol Cuminum cyminum extract and each of mentioned antibiotics in decimal assay of additivity ratio (0.5 for rifampicin and erythromycin and 0.5 for extract ,0.7 for enrofloxacin, and 0.3 for extract,0.6 for doxycycline, gentamicin and 0.4 for extract) against *S.aureus* isolates .**

**Key words**: *Antimicrobial combination, Staphylococcus aureus, cumin extract,* *antibiotics, synergism*.

**1. INTRODUCTION**

The emerging and sustained resistance to antibiotics and the poor pipeline of new antibacterial is creating a major health issue worldwide. Bacterial pathogens are increasingly becoming resistant even to the most recently approved antibiotics. Few antibiotics are being approved by regulatory organizations, which reflect both the difficulty of developing such agents and the fact that antibiotic discovery programs have been terminated at several major pharmaceutical companies in the past decade (Zapun *et al.,*2008 ).

 As a result, the output of the drug pipelines is simply not well positioned to control the growing army of resistant pathogens, although academic institutions and smaller companies are trying to fill that gap. An emerging option to fight such pathogens is combination therapy. Combinations of two antibiotics are emerging as a promising therapeutic approach(Cottarel and Wierzbowski, 2007).

New antibiotics were produced by pharmacological industries in the last three decades (White *et al*., 1993). Thus, it is extremely important to find new antimicrobial agents or new ways that are effective for the treatment of infectious diseases caused by drug-resistant bacteria(Taylor *et* *al*., 2002).

Few studies have found that the efficacy of antimicrobial agents can be improved by combining them with plant extracts against different multidrug-resistant pathogens (Ibezim et al., 2006 and Horiuchi et al, 2007).

There is a crucial and urgent need to develop new classes of antibiotics or to revitalize existing antibiotics (Tan et al., 2000).

Reasons that justify the use of antimicrobial combinations are broad-spectrum coverage for the initial therapy of severely infected patients, polymicrobial infections, and prevention of selection of resistant microorganisms when a high mutation rate of the causal organism exists to the antibiotic indicated, reduction of dose-related toxicity and antimicrobial synergistic activity(Acar, 2000).

Biological effects of these plants on prokaryotic and eukaryotic organisms have been discussed by few studies (Ababutain, 2011) . Because of their low toxicity, there is a long tradition of using dietary plants in the treatment of infectious disease in Cameroonian folk medicine (Djeussi *et* *al*., 2013).

In recent years, staphylococcosis has become one of the most important bacterial diseases of poultry. Chickens have been highly selected for their ability to achieve rapid growth. Such selection has added more stress by creating various leg problems that predispose poultry to the development of staphylococcosis. Historically, staphylococcosis has been a significant problem because of the ubiquitous nature of the bacterium in the poultry farm environment. When the door is left open, *staphylococcus aureus* has been able to localise the disease(Norton *et al*., 1994).

In this respect Awan *et al*. (2013)studied Chloroformic and isoamyl alcohol extracts of *Cinnnamomum zylanicum*, *Cuminum cyminum*, *Curcuma long Linn*, Trachy spermum ammi and selected standard antibiotics were investigated for their in vitro antibacterial activity against six human bacterial pathogens. The antibacterial activity was evaluated and based on the zone of inhibition using agar disc diffusion method. The tested bacterial strains were *Streptococcus pyogenes, Staphylococcus epidermidis, Klebsiella pneumonia, Staphylococcus aurues, Serratia marcesnces, and Pseudomonas aeruginosa*. Ciprofloxacin showed highly significant action against K. pneumonia and S. epidermidis while Ampicillin and Amoxicillin indicated lowest antibacterial activity against tested pathogens. Among the plants chloroform and isoamyl alcohol extracts of C. cyminum, S. aromaticum and C. long Linn had significant effect against P. aeruginosa, S. marcesnces and S. pyogenes. Comparison of antibacterial activity of medicinal herbs and standard antibiotics was also recorded via activity index. Used medicinal plants have various phytochemicals which reasonably justify their use as antibacterial agent.So the aim of this study is synergistic antimicrobial combination with Cumin extract with some antibiotics on *Staph.aureus* isolated from chicken.

**2. MATERIALS AND METHODS**

*2.1.Samples collection:*

A total 150 samples were aseptically collected from visceral organs (liver, gall bladder, spleen, kidney, cecum, heart and lung) of clinically diseased and dead chickens of different ages reared in farms located in Sharkia and Dakahlia governorates in period between 2014 till 2015.

*2.2. Bacteriological examination:*

*2.2.1. Isolation of Gram positive bacteria (Staph.aureus)*

In laboratory, the collected samples were transferred to sterile tryptone soya broth and incubated at 37 ºC for 24 hrs. A loopfull from each incubated broth was streaked onto the surface of mannitol salt agar, nutrient agar, blood agar, and baired Parker agar. The inoculated plates were incubated at 37°C for 24-48 hours and examined for bacteriological growth which appeared as yellow halo zone surrounding their growth on mannitol salt agar, smooth colonies had a low convex profile with an entire edge and pigmented yellowish on nutrient agar, zones of beta haemolysis which were clearly visible after incubation on blood agar and black, shiny ,convex colonies and surrounded by a clear zone of about 2-5mm in diameter on baired parker agar . One single colony which was showed typical colonial appearance and morphological characters was picked up and streaked onto semisolid agar media and was incubated at 37°C for 24 hours for further identification Koneman *et al*., (1992).

*2.2.2.Microscopic examination of Staph. aureus:*

Modified Gram's stain used as described by Cruickshank *et al*., (1975)

*2.2.3. Biochemical identification of Staph.aureus*

The methods of biochemical tests used for identification were carried out according to the schemes described by Koneman *et al*., (1992)

*2.3. Antimicrobial susceptibility testing*

Antimicrobial susceptibility test for all isolates was done according to description of Smith *et al.,* (1997)

*2.4. Preparation of methanol plant extract:*

The collected plant was cleaned from other contaminated plants &the fresh plant was collected and air dried away from sun light then the dried plants were crushed to powder using grinder. The powdered plant stored in tightly closed sterile containers until use Sooad *et al.,* (2012).

*2.4.2. Preparation of plant extract.*

Ten grams of powdered samples was dissolved in 100 ml of methanol in conical flask, plugged with cotton wool and then kept in rotatory shaker at 190-220 rpm for 24 hr. The extract was then filtered using conical flask with side arm, a filter funnel size (size 2) and a 90 mm diameter filter paper. Filtered extract was then poured in weighed 500 ml round bottom flask Solvent was evaporated with rotatory evaporator .Temperature of the water bath in the rotatory evaporator was set at 40 c .This temperature was used because the evaporation under reduced pressure makes it possible to evaporate at much lower temperature . Finally the extracts were preserved in sterilized dishes at refrigerator and to prevent from the light effect, they were wrapped with aluminum covers Sooad *et al.,* (2012).