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**THE IN VITRO EVALUTION OF CYMBOPOGEN  
CITRATUS (LEMONGRASS) ESSENTIAL OIL AS  
POTENTIAL ANTIMICROBIAL AGENT IN POULOTRY  
PRODUCTS**

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**ABSTRACT**

This study was carried out to investigate the potential of using essential oil extract of Lemongrass (*Cymbopogon citratus*) as natural antibacterial agent at three different concentrations (100, 200 and 500 X10<sup>3</sup> ppm) against 60 human isolates from diarrheal and septicemic cases in addition to 7 ATCC (American Type of Culture Collection) strains belonging to 3 different species of Gram negative bacteria including *Salmonella* spp, *Shigella* spp and *Campylobacter* spp and one Gram positive bacteria which is *Staph. aureus*. The screening was performed by standard disc diffusion method.

Essential oil extract of lemongrass exhibited maximum activity against *C. jejuni* with 30.62 mm, *Staph. aureus* with 11.91 mm, *Shigella* spp with 11.73 mm and *Salmonella* spp with 9.41 mm mean diameter of zone of inhibition at the lowest used concentration, 100x10<sup>3</sup> ppm of the essential oil.

Accordingly, lemongrass essential oil extract was found to be effectively inhibiting both gram-positive and gram-negative bacteria and can be a good source of antibacterial agents for possible infections in poultry products.

**Key words:** Lemongrass, antibacterial agents, *Salmonella*, *Shigella*, *Campylobacter*.

## INTRODUCTION

Poultry provide humans with companionship, food and fiber in the form of eggs, meat and feathers. Globally, production of the primary poultry products has been raising rapidly this reflects consumption based, in turn, on consumer preference for these high-quality products and the relatively low price because of efficiency of production. Over a 10-year period between 1995 and 2005, consumption, and hence production, has increased globally for chicken meat (53%), turkey meat (13%), duck meat (67%), goose meat (53%), chicken eggs (39%), and other eggs (27%) (Scanes 2007). Meanwhile, bacterial foodborne illness affects 48 million people in United States with 128,000 hospitalizations and 3,000 deaths (CDC, 2011). In addition to affecting millions each year, bacterial foodborne diseases are very expensive causing huge losses to the food industry and consumers. Despite methods to reduce or eliminate bacterial foodborne pathogens, food poisoning is still a major concern (Shan *et al.*, 2007). Due to increasing awareness among consumers and the increasing demand for "organic" foods free of synthetic additives and preservatives, the interest in developing novel antibacterial substances has increased (Suhaj, 2006).

The aim of this study is to use natural extract and compounds environmentally and healthy safe in the poultry slaughterhouses and industries to control some of microbial diseases that commonly occur in poultry which are *Salmonella*, *Shigella*, *Staphylococcus aureus* and *Campylobacter* isolates. Objectively, this study is to contribute information that will help to mitigate the microbial pollutants in poultry.

## MATERIAL AND METHODS

Bacterial strains: Sixty human blood and stool isolates of *Campylobacter* spp, *Shigella* spp, *Salmonella* spp and *Staphylococcus aureus*. In addition to seven reference strains of *Salmonella typhi*, *Shigella flexneri*, *Campylobacter jejuni*, *Campylobacter coli* and *Staphylococcus* were obtained from NAMRU-3 laboratory. Namely; *S. typhi* ATCC19430, *Staph. aureus* ATCC 25923, *Staph. aureus* (MRSA) ATCC 43300, *Campylobacter jejuni* ATCC 33291, *Campylobacter jejuni* (Invasive) ATCC 81176, *Campylobacter coli* ATCC 33559 and *Sh. flexneri* ATCC12022. All isolates and reference strains were stored at -80°C in brain heart infusion broth containing 15% glycerol (Pratt-Rippin and Pezzlo, 1992) since isolated as well as the reference strains were used for testing the quality of prepared media, reagents and spices oil extract discs used in this study. All bacterial isolates were recovered from preserved cryo-vials stored at -80°C using MacConkey plates that were incubated for 24 hours at 35±1°C and Blood plates that were incubated for 24 hours at 35±1°C with 5% Carbon Dioxide while the Skirrow plates that were incubated for 48 hours at 42°C in candle jars to maintain the microaerophilic atmosphere.

Lemongrass essential oil: Extract of *Cymbopogon citratus* (lemongrass) essential oil was gotten ready from Kanater station for essential oil production Agric. Res. Center, Egypt.

Antimicrobial activity screening: The antibacterial activity of essential oil extract was determined by the disc diffusion method as described by Clinical and Laboratory Standards Institute (CLSI) using the disc diffusion method by sterilized discs of filter paper which impregnated with 50 µL of essential oil extract dilutions of lemongrass before being placed on the

Mueller Hinton agar plates (100x15 mm) which inoculated with standardized inoculum ( $1.5 \times 10^8$  CFU/mL; 0.5 McFarland) of each freshly cultured of testing isolates and reference strains (*Salmonella* spp, *Shigella* spp, *Staphylococcus aureus*) suspended in sterile normal saline solution (0.85%) that was spread with the help of sterile cotton applicator. For *Campylobacter* spp, large plates of Mueller Hinton agar with blood were inoculated with standardized inoculum ( $3 \times 10^8$  CFU/mL; 1 McFarland) of each *Campylobacter* spp. using sterile cotton applicator. In addition to, negative control check embodied in sterile filter paper discs moistened with 50  $\mu$ L of each or combination of Tween-80 and Aqueous Dimethyl Sulfoxide (DMSO) at different concentrations. All inoculated plates of *Salmonella*, *Shigella* and *Staphylococcus aureus* containing the impregnated discs were incubated in an upright position at  $35 \pm 1^\circ\text{C}$  overnight and the inoculated plates of *Campylobacter* spp. containing the impregnated discs were incubated in an upright position in candle jar at  $42^\circ\text{C}$  for 48 hours. After incubation inhibition zone diameters were measured using digital caliper.

## RESULTS AND DISCUSSION

*Salmonella* spp, *Shigella* spp, *Campylobacter* spp and *Staph. aureus* were chosen as models for Gram-negative and Gram-positive bacteria because they are widespread serious foodborne pathogens in all food industry sectors and they pose increasing risks as by public health overseers (Archer, 1998; Bell and Kyriakides, 2002; Blackburn and McClure, 2002). The usage of antimicrobial resistant strains was suggested in our study because this provides more reliability and validity about the efficiency of examined

antimicrobial agents. These reference strains are invasive *Campylobacter jejuni* and Methicillin Resistant *Staphylococcus aureus* (MRSA). Both have shown reasonable results by giving a zone of inhibition 11.90 and 10.80 mm respectively, which can naturally control this kind of difficult organisms in poultry products.

Also, as shown in Table 1, the zone of inhibition for all types of bacteria significantly decreased with the increase of the essential oil concentration as a maximum activity against *C. jejuni* with 30.62 mm, *S. aureus* with 11.91 mm, *Shigella* spp with 11.73 mm and *Salmonella* spp with 9.41 mm mean diameter of zone of inhibition at  $100 \times 10^3$  ppm concentration of the essential oil. These inhibition zones have increased dramatically by increasing the essential oil concentration.

**Table 1:** Effect of different concentrations of Lemon -grass essential oil on different types of bacteria (mean diameter of zone of inhibition in mm).

Concentration (ppm)	<i>Campylobacter</i> spp	<i>Campylobacter jejuni</i> *	<i>Shigella</i> spp	<i>Salmonella</i> spp	<i>Staph. aureus</i>
$500 \times 10^3$	96.81	29.80	21.76	12.73	41.81
$200 \times 10^3$	51.21	15.70	16.87	10.06	31.82
$100 \times 10^3$	30.62	11.90	11.73	9.41	11.91

Invasive ATCC strain of *Campylobacter jejuni*

Pathogen control and decontamination measures significantly increase production costs. Moreover, contamination of food products with bacterial pathogens can seriously reduce consumer demand and affect producer profits (Pohlman *et al.*, 2002). The bio-preservative agent introduced in this study

was extracted from byproduct and inexpensive plant material which have economic importance for both producers and consumers.

In conclusion, since food safety is a major concern to the food industry and the consumers, research is ongoing constantly to find more effective methods to reduce or kill foodborne bacterial pathogens. In this study it was shown that the essential oil of lemongrass was effective in mitigate *Campylobacter jejuni*, *Shigella* spp, *Salmonella* spp and *Staphylococcus aureus* strains.

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## التقييم المعملی للزیت العطری لحشیفة الليمون كعامل مضاد للميكروبات المحتملة في منتجات الدواجن

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### المستخلص

تتهدد سلامة الأغذية من قبل العديد من مسببات الأمراض التي تسبب العديد من الأمراض المنقولة عن طريق الغذاء التي تسبب الأمراض الحادة وقد تكون السبب في بعض الأمراض المناعية والكلية وغيرها. لذا أجريت هذه الدراسة للتحقق من إمكانية استخدام الزيت العطري لنبات حشيشة الليمون (*Cymbopogon citratus*) كمصدر طبيعي مضاد لبعض الكائنات الحية الدقيقة المسببة للمرض من خلال ثلاثة تركيزات مختلفة (١٠٪، ٢٠٪ و ٥٠٪) ضد ٦٠ عزلة من مرضى يعانون من الإسهال أو التسمم بالدموى بالإضافة إلى ٧ سلالات مرجعية ينتمون إلى ٣ أنواع مختلفة من البكتيريا سالبة لجرام شملت *Salmonella spp* و *Shigella spp* و *Campylobacter spp* ونوع آخر موجب لصبغة الجرام وهو *Staph. aureus*. تم إجراء الفحص باستخدام طريقة الانتشار بالأقراص.

أظهر الزيت العطري لحشيشة الليمون أقصى نشاط له ضد بكتريا *C. jejuni* ٣٠,٦٢ ملم، *S. aureus* ١١,٩١ ملم، *Shigella spp* ١١,٧٣ ملم و *Salmonella spp* ٩,٤١ ملم كمتوسط قطر منطقة التثبيط عند أقل تركيز مستخدم  $100 \times 10^3$  جزء في المليون من الزيت العطري. وعليه تم التوصل إلى أن الزيت العطري لحشيشة الليمون اظهر فعالية عالية ضد كلاً من البكتيريا الموجبة والسالبة لصبغة جرام، ويمكن أن يكون مصدراً جيداً كمصدر طبيعي مضاد للكائنات الحية الدقيقة يمكن استخدامه في حفظ بعض المنتجات الغذائية خاصة منتجات لحوم الدواجن.