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# Effect of clove and cinnamon oils on Escherichia coli isolated from chicken meat

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**Abstract**
Chicken is the most widely accepted meat in India. Poultry meat and eggs are rich and pure source of high quality balanced nutrient including proteins, vitamins, and minerals with excellent biological value. The bacterial populations were enumerated on chicken meat which collected in different location and control of *E. coli* by clove oil and cinnamon oil. Chicken meat samples were collected from the different slaughtering shop which located at roadside outlets, non-roadside outlet and modernized slaughtering unit. The high microbial load was found in the sample collected from roadside outlets followed by non-roadside outlets, and modernized slaughtering unit. The contamination level of total coliforms and fecal coliforms were ranged between 3.08±0.0602 and 5.38±0.0305 and 1.79±0.0950 and 4.28±0.0435 respectively. *E.coli* was highly susceptible to clove oil (25 mm) than cinnamon oil (11mm). So the use of essential oil to wash or sanitize chicken meat will reduce the number of most harmful pathogens and microbial loads on meat carcasses, which will increase the shelf life and meat quality.

**Keywords:** essential oils, poultry, coliforms, *e.coli*, mesophiles, psychrotrophs

## Introduction
The Poultry Business in India is a very old practice and this food industry is one of the important contributors to the economy of rural and semi-urban India. Further, India is the fifth largest producer of eggs and ninth largest producer of poultry meat amongst all the countries (Ravichandran and Khan Mohamed, 2015) [15]. Contamination of poultry meat with foodborne pathogens remains an important public health issue, because it can show the way to illness if there are malpractices in handling, cooking or post-cooking storage of the product. Total count of aerobic mesophilic bacteria, Enterobacteria and *Escherichia coli* are considered indicators of microbiological quality (Capita *et al.*, 2002) [4]. Total count of aerobic mesophilic bacteria in ground chicken meat is always high and consequently, the risks of spoilage in the sense of microbiological disintegration are higher.

Recently there has been an increasing interest in discovering new natural antimicrobial substances or bio preservatives for the preservation of meat and meat products. Bio-preservatives include a range of natural products from plants, animals and microorganisms which can be used to improve the keeping quality of foods. Being plant natural food stuffs, spices appear to be an alternative for the chemical antimicrobials to the consumers who tend to question their safety. Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties.

Essential oils are a rich source of biologically active compounds. There has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants particularly essential oils. Hence the essential oils of spices or bio preservatives have the greatest advantage to cater to the demands of the consumer for the natural preservatives. Garlic, clove and cinnamon are some of the most commonly used ingredients as a flavour enhancer for foods. In addition to flavouring the foods, these spices are appreciated for their medicinal properties. Clove and cinnamon have a wide spectrum of actions not only antibacterial, anti-viral, antifungal and antiprotozoval, but also have beneficial effects on the cardiovascular and immune system (Harris *et al.*, 2001).

## Materials and Methods
### Sample Collection
Fifty gram samples of chicken meat were collected from different slaughtering shop which located at roadside outlets and non-roadside outlets with moderate facilities. The samples were transported in ice at 4ºC to laboratory till processed for microbial analyses.

**Sample Preparation** (Sengupta *et al.*, 2012) [16]
Ten gram of meat sample was weighed and transferred in sterilized mortar and minced in sterilized Ringer's solution with the help of sterile pestle and then it is serially diluted up to $10^{-5}$ were prepared for the microbiological analysis.

### Microbiological Analysis
**Total Mesophiles and Psychrotrophs Count** (Chaiba *et al.*, 2007) [5]
Mesophiles and psychrotrophs were determined using plate count agar and spread plates were incubated at 30 and 8°C for 72 hr. respectively.

**Total Coliforms and Fecal Coliforms Count** (Chaiba *et al.*, 2007) [5]
For enumeration of coliforms, the pouring plate technique was used. Deoxycholate Lactose Agar (45– 50°C) was poured into 0.1 ml of inoculum. The plates were incubated at 37°C for enumeration of total coliforms and at 44°C for enumeration of fecal coliforms for 24 - 48 hr. All typical colonies (red colonies) were counted and the organisms were

determined by the spread plate method using eosin methylene blue (EMB) agar for total *Escherichia coli* count. The isolated colonies were identified morphologically and biochemically.

**Data Analysis**

The diversity indices of the bacterial population of each study site were analyzed separately using Biodiversity Pro software (McAleece *et al*., 1997) [13].

**Evaluation of Plant Essential Oil for Their Antibacterial Activity**

**Disc Diffusion Method**

The essential oils such as clove oil and cinnamon oil were collected and oil sensitivity testing was done for all the isolates on Mueller-Hinton agar by modifying Kirby-Bauer disc diffusion technique (Bauer *et al.*, 1966) [2].

**Minimal Inhibitory Concentration (Mic) of Essential Oil**

Minimal Inhibitory Concentration (MIC) of organic acids tests were carried out by Eloff (1998) [7] method with modification. The stock solutions of the essential oil ( 50%) were diluted and transferred into the first well of micro titre plate and serial dilutions were performed and 0.75 µl was added to each tube so that the concentrations in the range of 50 to 0.024 % of essential oil and 50 to 0.024 µg/ml of amikacin were obtained. The double strength nutrient broth (0.75 µl) and 20 µL of the inoculum were added to all tube which were incubated at 37°C for 24 h. MIC was detected by adding 20 µl of 0.002% TTC (Triphenyl Tetrazolium Chloride, Loba). The colourless tetrazolium salt was reduced to a red-coloured product by the biological activity of the organisms. The contents of the tube turned to red or purple if any microbial growth was present. MIC was defined as the lowest concentration of essential oil that inhibited visible growth, as indicated by the TTC staining.

**Dipping Procedure (**Bin Jasass, 2008) [3]

For each treatment, 4 chicken quarters without skin, obtained from a chicken stall were used. These samples were dipped into the suspension of *E. coli* -3 ($10^7$ CFU/ ml) for 20 second and put in a tray for 90 min. to allow *E. coli* to be attached to the surface of chicken meat. The samples were subjected to one of the following treatments: 0.5, 1% and 2% concentrations of essential oil for 20 second. Then all samples were also dipped in sterile water for 20 second.

**Surface Swabbing** (Bin Jasass, 2008) [3]

A sterile template (4 x 4 cm) was put on chicken surface, and the area was swabbed by sterile swab cotton. The swab was put in 9 ml of 0.1% peptone. Decimal dilution series were prepared and were spread plated (0.1 ml) in duplicate on selective agar in the pH 7.0 for enumeration of *E. coli.* Plates were incubated at 37ºC for 24 hr and results were calculated log colony forming unit and tabulated.

**Statistical Analysis**

Means with standard error computed with help of IBM SPSS Statistics-20 (SPSS Inc., IBM Company Chicago, IL, USA, 2010).was used for statistical analysis

**Result and Discussion**

The total mesophiles mean log values in roadside roadside outlets were higher [6.08±0.0608] than non-roadside outlets and modernized slaughtering unit were. The psychrotrophs count of samples were also higher4.82±0.0300 in roadside outlets, than non-roadside outlets and modernized slaughtering unit which were similar to those noted by Fliss *et al.* (1991) [8] and Chaiba *et al*. (2007) [5].

The contamination level with total coliforms of samples collected from slaughtering shop in roadside outlets, non-roadside outlets and modernized slaughtering unit was 5.38±0.0305, 5.04±0.0556 and 3.08±0.0602 $\log_{10}$ cfu/g respectively. For fecal coliforms average counts were 4.28±0.0435, 3.15±0.0550 and 1.79±0.0950 $\log_{10}$ cfu/g in samples purchased from slaughtering shop in roadside outlets, non-roadside outlets and modernized slaughtering unit respectively. Total *Escherichia coli* count in chicken meat slaughtering shop in roadside outlets, non-roadside outlets and modernized slaughtering unit was 3.72±0.1205, 2.76±0.6996 and 1.04±0.2510 $\log_{10}$ cfu/g respectively. Datta *et al*. (2012) reported that the total coliform count in meat samples 1, 3, 4, 6 and 8 were 4.31, 4.42, 5.5, 5.58, and 4.8, respectively. None of the total coliform count pathogens were noticed in samples 2, 5, 7, 9 and 10. The roadside outlets and non roadside outlet were wide difference with modernized slaughtering unit. The Shannon diversity index H` varied between 0.298 and 0.511. Shannon evenness index J` is in the range between 0.426 and 0.732.The relative abundance of the microbial population was measured using Simpson, Berger-Parker and McIntosh indices. The Simpson index value ranged from 0.223 to 0.643 and the Berger-Parker index varied from 0.345 to 0.785.

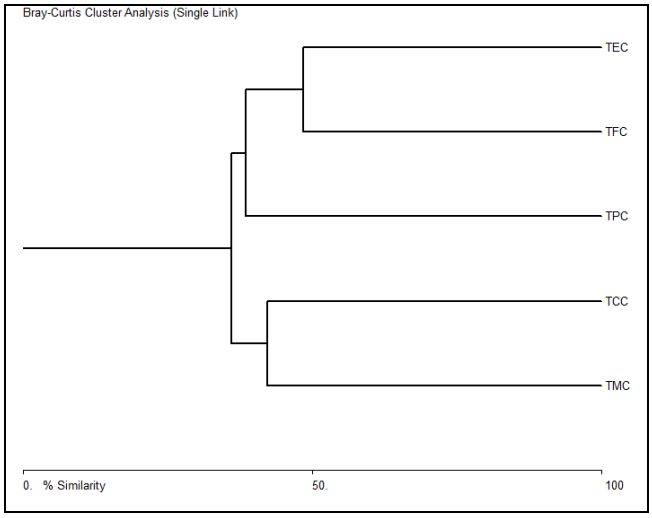


**Fig 1:** Bray- curtis cluster analysis of different type of bacteria isolated in three locations

The Mackintosh index ranged between 0.502 and 0.991. Although Mackintosh Diversity (D) and Mackintosh Eveness (E) values for different study sites were almost all of equal magnitude. In the Simpson index (D), 0 represents infinite diversity and 1, no diversity, that is, the greater the value of D, the lower the diversity but the reverse is true in the case of Berger -Parker and Shannon-Wiener indices (Ludwig and

Reynold, 1988; Mateus *et al*., 2006) [11, 12]. Five isolates of *E.coli* was identified based on morphological and biochemical characterization and these isolates were used for further studies. The results of the disc diffusion test revealed that the essential oil of clove and cinnamon showed different degrees of growth inhibition, depending upon the bacterial strains. *E.coli* was highly susceptible to clove oil which produced 14 mm zone of inhibition to 25 mm. The compared with clove oil, cinnamon oil had a low zone of inhibition which ranged between 8-11 mm. (Table 1). The MIC value of clove oil was ranged between 0.39 and 3.13% while cinnamon oil between 12.5 and 25 %. (Plate-1)

**Table 1:** Antibacterial activity of plant essential oil against *E.coli*

| S. No. | Essential oils | Diameter of inhibition zone (mm) | | | | |
|---|---|---|---|---|---|---|
| | | ***E.coli*-1** | ***E.coli*-2** | ***E.coli*-3** | ***E.coli*-4** | ***E.coli*-5** |
| 1 | Clove oil | 20 | 23 | 14 | 25 | 20 |
| 2 | Cinnamon oil | 8 | 10 | 8 | 11 | 9 |
| 3 | Amikacin 30 mcg | 18 | 20 | 17 | 20 | 19 |

The results of this study were in agreement with Moreira *et al.* (2005) [14], who observed the significant bactericidal action of clove against Escherichia coli. Helander *et al.* (1998) [10] who observed the inhibition of Escherichia coli O157:H7 and Salmonella typhimurium by the essential oil of cinnamon. The presence of complex chemical structures constituted of several groups, such as terpenes and terpenoids, aromatic and aliphatic constituents, all characterized by low molecular weight, may explain their successful bacteriostatic and bactericidal action Bakkali *et al.*, 2008) [1].

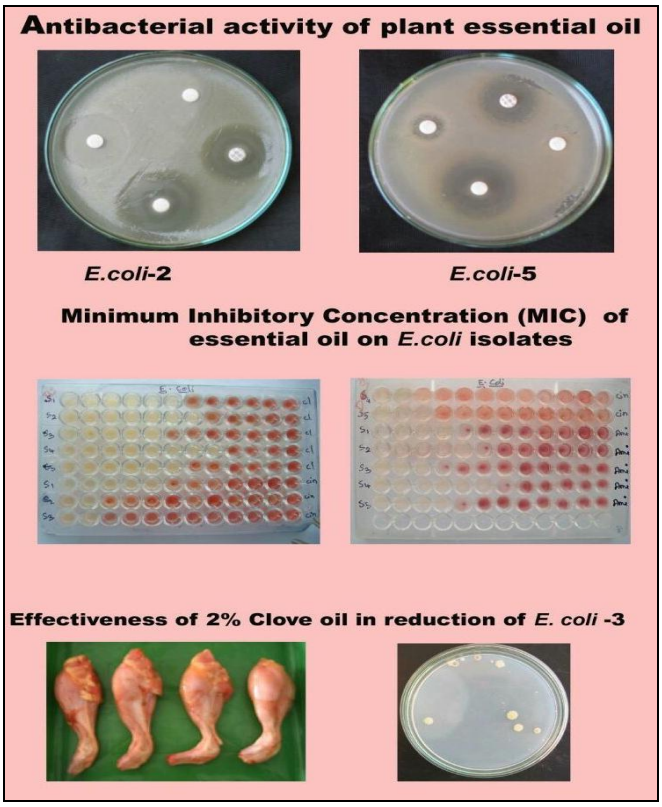


**Fig 1**

Among the five isolates of *E.coli*, isolate *E.coli* -3 had the highest resistance to essential oil. This isolates selected for reduction of the population on chicken surface experiments. In the present study, clove oil eradicated the *E.coli* 34.64 % at 0.5% concentration while 57.68 at 1% concentration and 88.68% at 2% concentration on chicken meat surface. In the case of cinnamon oil, 18.60 % at 0.5% concentration, 26.95 at 1% and 57.95 % at 2% concentration on chicken meat surface. The presence of protein or fat in foods could protect food from the effect of essential oils. Therefore, higher concentrations of EOs are needed to effectively control the microorganism in food compare to in vitro studies.

## Conclusion

As the food industry is facing great challenges to produce safe, and at the same time food without synthetic chemical preservatives, essential oils make their way into the scientific focus. Due to their antibacterial, antifungal and antiviral activity, as well as antioxidant properties, they are used to prevent food borne diseases, to extend shelf-life, and to improve some meat characteristics. Essential oils are recognized to be used, not only as food additives, but also in aromatherapy, antitumor therapy, as potential antimicrobial agents against multi-resistant bacteria, and in other purposes in medical and nonmedical fields. Yet, benefits of their use remain to be confirmed.

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