# Decontaminating effect of *Neolitsea cassia* leaves extract on broiler chicken meat contaminated with *Salmonella typhimurium* and *Escherichia coli*

**M.M.D.N.H. Gunathilake, S.U. Pathiranage, D.N.N. Madushanka, T.S.P. Jayaweera, H.A.D. Ruwandeepika***

*Department of Livestock Production, Faculty of Agricultural Sciences, Sabaragamuwa University of Sri Lanka, P.O. Box 02, Belihuloya, Sri Lanka*
** ruwandeepika@yahoo.co.uk*

## 1. Introduction

Unsafe food leads to severe losses, estimating 600 million foodborne disease cases annually at global scale due to the consumption of contaminated foods with 420 000 deaths/year. Foodborne diseases are mainly associated with the consumption of meat and meat products contaminated with pathogenic microorganisms such as bacteria, viruses and parasites. *Salmonella* spp., *Campylobacter, Listeria* spp., *Clostridium* spp., *E. coli* and *Staphylococcus* spp. are the most common bacteria leading to foodborne disease outbreaks affecting millions of people annually. These outbreaks leads wide range of symptoms from severe to fatal outcomes. As the treatment options, antimicrobials are used and they are also used in sub-therapeutic levels to maintain animal and human health. This indiscriminate use of antimicrobials has resulted in a global threat, i.e. emergence of antibiotic resistant. More importantly, resistant bacteria can be transmitted to human through the food chain. In order to address this globally important threat of antibiotic resistance, scientists are in search of alternatives for antibiotics. Use of natural antimicrobial agents has received much attention as it is one of the best solution for the above problem. Therefore, application of plant based natural antimicrobials could be better option for the synthetic antibiotics and hence reducing the development of antibiotic resistance. *Neolitsea cassia* is a plant of Lauraceae family and known to have some beneficial effect such as antifungal, antioxidant and etc. However, there are hardly any researches so far on investigating the antibacterial properties, antioxidant properties, anti-biofilm properties, cytotoxicity of *N. cassia*. Hence this study investigated the decontamination ability of aqueous extract of *N. cassia* leaves on broiler chicken meat contaminated experimentally with *Salmonella* Typhimurium and *E. coli.*

## 2. Materials and Methods

Decontamination ability of the aqueous extract of *N. cassia* leaves on broiler chicken meat contaminated experimentally with *S.* Typhimurium (ATCC 14028) and *E. coli* (ATCC 25922) was investigated according to the method described by Madhushanka et al. (2018). Bacterial cell suspensions were prepared at $1.0 \times 10^7$ colony forming units per ml (CFU/ml) following the McFarland 0.5 turbidity standard. Aqueous extract of *N. cassia* was collected by passing through sterilized cheese cloth under aseptic conditions and this extract was used for meat decontamination. Chilled chicken meat samples (each sample weighing, 2.5g±0.2 g) were sterilized using sterilized distill water and subsequent UV treatment. Meat samples were artificially contaminated with the concentrations of $10^7$ CFU/g of *S.* Typhimurium and *E. coli* under sterilized conditions and then were shaken in an automatic shaker for 30 minutes at 100 rpm/minute. Meat samples without contamination with *Salmonella* and *E. coli* were used as the uncontaminated control to see the sterility of meat samples. Three different concentrations (500mg/ml, 250mg/ml, and 125mg/ml) of aqueous extracts of *N. cassia* were used as treatments. Meat samples that were contaminated with bacteria were separately dipped in

different concentrations of aqueous solutions for two dipping times as 15 and 30 minutes in mechanical shaker at 100rpm. Contaminated meat samples without treatment of aqueous extract was taken as the positive control. Each treatment had six replicates. Decontaminated meat samples were homogenized and serially diluted and enumerated on Hektoen enteric agar (for *Salmonella*) and McConkey agar (for *E. coli*) plates using spread plate method. Following the incubation at 35±2°C for overnight, colonies were counted using automatic colony counter. Data were analyzed using the SAS statistical software.

## 3. Results and Discussion

This study investigated the decontamination effect of N. cassia extract on meat contaminated with S. Typhimurium (ATCC 14028) and *E. coli* (ATCC 25922).

### 3.1 Effect of *Neolitsea cassia* on chicken meat contaminated with *Salmonella* Typhimurium (ATCC 14028)

This study revealed a higher reduction of S. typhimurium count in meat samples dipped at higher concentration (500mg/ml) of the N. cassia than that of meat samples dipped in lower concentration (250mg/ml) for the both the dipping times (15 and 30 minutes). Percentage reduction of *S.* Typhimurium in the meat samples dipped at the concentration of 500mg/ml concentration level was 54% and 62.9% respectively for 15 and 30 minutes dipping time when compared to the concentrations of 250mg/ml and 125mg/ml. Percentage reduction of *S.* Typhimurium was 50.8% & 58.8% in the meat samples dipped at the concentration of 250 mg/ml and it was 125 mg/ml concentration levels 13.8% and 30.6% for15 minutes and 30 minutes dipping time respectively (Table 01).

**Table 01. Effect of different concentrations of aqueous extract of *Neolitsea cassia* on count of *Salmonella typhimurium* in contaminated meat samples**

| Treatments | 15 minutes | | 30 minutes | |
|---|---|---|---|---|
| | Average colony count in log 10 | % Reduction | Average colony count in log 10 | % Reduction |
| Positive control | $5.169^{g}$ ± 0.01 | 0 | $5.216^{i}$ ± 0.02 | 0 |
| 500mg/ml | $4.832^{h}$ ± 0.02 | 54.0 | $4.785^{k}$ ± 0.02 | 62.9 |
| 250mg/ml | $4.861^{h}$ ± 0.02 | 50.8 | $4.830^{k}$ ± 0.02 | 58.8 |
| 125mg/ml | $5.102^{g}$ ± 0.06 | 13.8 | $5.056^{j}$ ± 0.03 | 30.6 |

*Data were presented as the mean ± standard deviation. Means with different superscripts in the same column are significant differences at ($p \leq 0.05$)

Findings of Tabak et al. (1999) also in line with the results, as the concentration of the *N. cassia* at 125mg/ml may be supply enough nutrients for microbial survival but low enough to exert an inhibitory effect on *S. typhimurium*. The results prove that with the increment of concentration of *N. cassia* aqueous extract, the colony count reduction has been increased. This can be proved through the findings of the Piskernik et al. (2010).

### 3.2 Effect of *Neolitsea cassia* on chicken meat contaminated with *E. coli* (ATCC 25922)

The experiment of meat contamination with *E. coli* (ATCC 25922) also exhibited reduction of the bacterial cell numbers by 59.4% and 63.4% in the meat samples which were immersed in the 500mg/ml concentration of *N. cassia* extract for both dipping times 15 & 30 minutes respectively. The second highest percentage reduction of *E . coli* cell number was at 250mg/ml concentration and the reduction percentages was 57.7% and 59.6% respectively for both

dipping times and the lowest percentage reduction of bacterial cell numbers was by 125mg/ml and the reduction was 37.5% and 47.5% respectively for both the dipping times (Table 02).

**Table 02. Effect of different concentrations of aqueous extract of *Neolitsea cassia* on count of *E. coli* in contaminated meat samples**

| Treatments | 15 minutes | | 30 minutes | |
|---|---|---|---|---|
| | Average colony count in log 10 | % Reduction | Average colony count in log 10 | % Reduction |
| Positive control | $5.204^{a}$ ± 0.01 | 0 | $5.247^{d}$ ± 0.02 | 0 |
| 500mg/ml | $4.813^{c}$ ± 0.01 | 59.4 | $4.810^{f}$ ± 0.02 | 63.4 |
| 250mg/ml | $4.830^{c}$ ± 0.02 | 57.7 | $4.853^{f}$ ± 0.01 | 59.6 |
| 125mg/ml | $5.000^{b}$ ± 0.06 | 37.5 | $4.966^{e}$ ± 0.03 | 47.5 |

*Data were presented as the mean ± standard deviation. Means with different superscripts in the same column are significant differences at ($p \leq 0.05$)

A study done with cinnamon extract against *E. coli* has been shown the possibility of controlling the *E. coli* by these natural compound (Bharath et al., 2016) and it further supports the findings of this study too.

Phytochemical analysis had been proved that dried mucilaginous material of *N. cassia* contained with carbohydrate, monosaccharide, tannins, flavonoids and alkaloids (Kusunmala et al., 2017; 2019). But aqueous leaf extract has been contained with arabinose and xylose together with small amount of other sugars. The high viscosity of the *N. cassia* aqueous leaf extract is having due to water soluble arabinoxylan (De Silva & Kumar, 1986). Therefore, mucilaginous material which is contained in *N. cassia* leaf extract is shown antibacterial effect on S. typhimurium and *E. coli* treated chicken meat as above result.

Further this study revealed that the percentage reduction was not significantly affected ($p<0.05$) by dipping time but there was biological effect with elevated dipping time; some increment of percentage reduction was at 30 minutes dipping time in all three concentration levels in both the bacterial species used.

Uncontaminated control (sterile meat samples without contamination and without treatment with extract) did not exhibit the growth of microorganisms as it was free from microorganisms of concern.

## 4. Conclusions

This study concluded that there was a significant decontamination effect in aqueous extracts of *N. cassia* against *Salmonella* and *E. coli* at varied levels of concentration.

## 5. References

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