

# CAMELLIA SINENSIS AS A NATURAL MEAT PRESERVATIVE IN ASSOCIATION WITH ITS TOTAL POLYPHENOL CONTENT

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**Abstract:** *Camellia sinensis* (Tea) is one of the most popular nonalcoholic beverages, consumed by over two-thirds of the world's population. The aim of the present study was to establish a co-relation between the polyphenol content and antimicrobial activity of green tea. In the former part of the research, the total polyphenol content of the same varieties was checked and were found to be  $19 \pm 0.866\%$ ,  $5.3 \pm 0.288\%$  and  $23 \pm 0.763\%$  respectively. The test organisms used were spoilage organisms from chicken as *S.aureus*, *E.coli*, *Bacillus spp* and *Salmonella typhi*. The antimicrobial activity of three different specimens of green tea samples viz. Lipton tea (commercial processed tea), Organic India green tea (organic tea) and Tenfu tea (Chinese tea) was checked against the standard. The Lipton tea was found to be most inhibitory towards the organisms and the least inhibitory was Organic India Green tea; their Mean zone of inhibition being  $9.5 \pm 2.08$  mm and  $2.25 \pm 4.5$  mm respectively at 5mg/ml. the coefficient of correlation(r) was found to be 0.92 which indicated that the antimicrobial activity of green tea is directly proportional to its total polyphenol content.

**Keywords:** *Camellia sinensis*, Green tea, Antimicrobial agents, Total polyphenol content.

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## I. INTRODUCTION

The most common perishable foods to be spoiled are the foods that have high water activity like fruit juices and those that have high amounts of nutrients like milk and milk products, animal products and cereals (Gustavsson, Cederberg and Sonesson, 2011). Poultry meat being a nutrient dense food product is therefore highly susceptible to spoilage from microbes like *Bacillus subtilis* which can metabolize proteins with the help of protease enzyme and convert them to polypeptides and further amino acids (Khan et al., 2011). The common spoilage organisms related to poultry are *Brucella*, *Mycobacterium tuberculosis*, *Coxiella*, *Listeria*, *Campylobacter*, beta-hemolytic *Streptococci*, Enter pathogenic *E.coli*, *Staphylococci* and *Salmonella*, parasites and viruses (Frazier and Westhoff, 1999).

A variety of physical preservation techniques like low temperature storage, freezing, radiation and modified atmosphere packaging (Zhou, Xu and Liu, 2010) as well as chemical preservation techniques like using alcohols, phenols and nitrates and nitrites are used to preserve poultry meat (Jay, 2000). The drawback of these methods is that they are costly and could be inefficient to inhibit the microbes completely; hence, antibiotics/ chemical antimicrobials are used for preventing the spoilage. The overuse of antibiotics has led to the rise of antibiotic resistant strains. Daglia (2012), considers an emerging urgent need for a new antimicrobial agent as very urgent due antibiotic resistance shown by major organisms.

Tea is one of the most popular nonalcoholic beverages, consumed by over two-thirds of the world's population because of its refreshing, mild stimulant and medicinal properties. Green tea polyphenol like catechins have shown some protection

against the *Streptococcus mutans* which cause dental infections in humans, improved gut bacteria and also helps to reduce the microbial load during cancers like reduction in population of *H.pylori* during gastric cancer (Afaq et al., 2004). Also, antimicrobial effects of green tea catechins and polyphenols can help in protection from microbial load during vaginitis (Kim et al., 2013) along with anti-aflatoxin activity on *Aspergillus flavus*. Also, Gong (2006), described the use of tea polyphenol on the antibiotic resistant *S.aureus*. Begoun (2004), explains that tea polyphenols have an ability to disrupt the selective permeability of cell membranes thus leading to changes in the osmotic functions of the cells which leads to cell death. A study done Hz (2013), showed inhibitory effects of different Chinese traditional teas on aflatoxin (the most carcinogenic substance) from the *Aspergillus flavus* organism which gave positive results on the toxin but not on the mycelial growth of the organism. The mechanism that they found was due changes in transcription of the major aflatoxin proteins.

The present study aims at studying the correlation between polyphenol content and corresponding antimicrobial effect of different varieties of green tea.

## II. MATERIALS AND METHODS

Estimation Of Total Polyphenol Content Of The Green Tea Samples Using Gallic Acid As Standard And Folin-Ciocalteu Reagent (Iso14502-1).

### II.I Reagent Preparation

The reagents required were 70% methanol (70 ml methanol + 30 ml distilled water), 7.5 % sodium carbonate solution (7.5 grams sodium carbonate in 100 ml distilled water) and 10 % Folin Ciocalteu reagent (10 ml FC reagent + 90 ml distilled water.)

### II.II Stock Preparation

0.1g of Gallic acid was weighed and diluted to 100 ml. this stock solution was diluted with distilled water to obtain concentration range from 10 µg/ml - 50 µg/ml of Gallic acid.

### II.III Sample Preparation

0.2 g of sample was weighed and kept in extraction tube. 5 ml of 70% methanol was added and kept it in water bath for 10 min set at 70° C. The samples were mixed at regular intervals on vortex mixture for proper extraction. Centrifuged samples at 3500 rpm for 5 min were transferred in other tubes. The volume is made to 10ml with 70% methanol. 1 ml from this sample is taken and diluted to 100 ml with distilled water. This was the final sample.

### II.IV Colorimeter Procedure

To 1 ml of sample tubes 5 ml of 10 5 FC reagent and 4 ml of 7.5% sodium carbonate solution. Appearance of blue colour confirmed the presence of polyphenol. The reading for absorbance was taken at 660 nm.

The total polyphenol content is calculated as

$$\text{Total polyphenol content} = \frac{\text{O.D (sample)} \div \text{O.D (standard)}}{\text{X dilution factor (100)}} \times \text{Concentration of Standard}$$

## III. ANTIMICROBIAL EFFECT OF GREEN TEA SAMPLES

### III.I Sample Preparation

5 grams of three green tea samples (Lipton green tea, Organic India and Tenfu tea) were taken in 100 ml of distilled water. Each of the samples was boiled for 5 minutes and 10 minutes separately. The concentration of the stock solution was thus 5 mg/ml.

### III.II Paper Diffusion Assay

The laboratory cultures of the spoilage organisms which were identified in the previous step were used to assess the antimicrobial effect of the above mentioned green tea samples by the paper diffusion method. The spoilage organisms were streaked on Nutrient agar plates. Sterile discs of What Mann filter papers were dipped in the tea samples and then

placed on the swabbed plate. After incubation for 24 hours at 37 °C, the zone of inhibition for the tea samples were checked.

Tube no.	Stock Concentration (µg/ml)	O.D of standards (660 nm)
1	10	0.16
2	20	0.32
3	30	0.46
4	40	0.59
5	50	0.78
	Samples	O.D of standards (660 nm)
Sample A	Set I	0.58
Sample A	Set II	0.54
Sample A	Set III	0.58
Sample B	Set I	0.19
Sample B	Set II	0.16
Sample B	Set III	0.18
Sample C	Set I	0.76
Sample C	Set II	0.71
Sample C	Set III	0.73

*Results: Total polyphenol content*

Table No.1: O.D of Standard Gallic Acid and Polyphenol Extract Of Green Tea Samples At 660 Nm

Samples	Total polyphenol content (%) expressed as Gallic Acid Equivalents			Mean ± S.D total polyphenol content (%)
	Set 1	Set 2	Set 3	
A	19.5	18	19.5	19 ± 0.866
B	5.5	5	5.5	5.3 ± 0.288
C	24	22.5	23	23 ± 0.763

Table No 2: Total Polyphenol Content of Green Tea Samples

**III.III Paper Diffusion Assay**

The green tea samples viz., Lipton green tea, Organic India green tea and Tenfu tea were labeled as sample A, B and C respectively. The antimicrobial sensitivity for the standard laboratory cultures of spoilage organisms from chicken *S.aureus*, *E.coli*, *S.typhi* and *Bacillus spp* was checked using paper diffusion assay. The concentration of the tea sample taken was 5 mg/ml.amongst the three green tea samples, Sample A showed greatest inhibition against the standard cultures.

#### IV. DISCUSSION

Coefficient of correlation (Pearson's method) was used to check the correlation between the mean polyphenol content and the zone of inhibition for the respective tea samples the r value obtained was 0.92 which indicated that there is a correlation between the two variables. So it can be stated that the higher the polyphenol content more will be the antimicrobial effect and vice versa. The results obtained in the present study indicate that green tea possessed possible antimicrobial properties on spoilage organisms due to its high polyphenol content. Green tea could thus be used as a preservative in poultry meat as premarinating agent to increase its shelf life.

#### V. CONCLUSION

The aim of the present study was to determine the antimicrobial effect of green tea on spoilage organisms in chicken and to establish its relation with the polyphenol content of the same green tea samples. Also, Green tea showed significant antimicrobial activity against all the spoilage organisms found in chicken meat. There was an evident direct proportion between the observed antimicrobial effect and the calculated polyphenol content. Thus, it can be concluded that green tea, a rich source of plant polyphenol is an effective antimicrobial agent for the control of spoilage in chicken meat. Green tea can thus be used as a novel natural preservative agent for chicken meat.

	Tea samples	Sample A (5mg/ml)		Sample B(5 mg/ml)		Sample C (5mg/ml)	
		5'	10'	5'	10'	5'	10'
	<b>Boiling time (minutes)</b>						
		<b>Zone of inhibition(mm)</b>					
<b>Cultures</b>							
<i>E.coli</i>		7	6	0	0	8	8
<i>Bacillus</i>		10	9	9	0	9	10
<i>S.aureus</i>		9	10	0	0	6	8
<i>S. typhoid</i>		12	13	0	0	10	7
	Mean ± S.D.	9.5 ± 2.08	9.5 ± 2.88	2.25 ± 4.5	0 ± 0	8.2 ± 1.2	8.2 ± 1.2

Table No. 3: Zone Of Inhibition of Green Tea Samples

#### VI. RECOMMENDATIONS

Different polyphenol components can be extracted and their antimicrobial activity can be compared to determine which one is most potent. A shelf life study to check the extent to which green tea can be used to preserve chicken meat can be done.

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