



# Usage of Phenolic Bay Laurel Extract as Microbial Agent to Preserve Chicken Meat

Dima Al Diab, Nazih Daood, Ahmad Ahmad



**Abstract:** The purpose of this study is to evaluate the antimicrobial effect of bay laurel extract (*Laurus nobilis*, *L. nobilis*) on chicken meat during storage for 15 days at a temperature 4°C. Chicken meat was treated either with doxycycline (positive control) or with bay laurel extracts (100ppm, 300ppm, 900ppm), negative control was chicken meat without any treatment. The Folin measured total phenolic content- ciocalteu colorimetric method. The total plate count (TPC) of bacteria was monitored after the days (1-7-14) of treatment. All extracts showed antimicrobial activity against the tested bacteria, but the 900-ppm concentration was most effective for extending the shelf life of chicken meat.

**Keywords:** Total Phenolics, Total Plate Count, Antimicrobial, Laurel. *Nobilis* Extract, Chicken Meat.

## Nomenclature:

NA: Nutrient Agar

GAE: Gallic Acid Equivalents

TPC: Total Plate Count

## I. INTRODUCTION

Health information and food-safety data are widely available, thereby meeting consumer demand for safe, high-quality food [1]. Chicken meat is preferred by consumers worldwide because of its desirable nutritional profile, low-fat content, and moderate concentration of polyunsaturated fatty acids [2]. Fresh meat is usually marketed refrigerated at a temperature (2-5 °C), but Bacterial growth and fat oxidation may occur during storage [3], causing spoilage of meat. In addition, chicken meat is contaminated with microbes during slaughter and industrial processing, where the growth of these microbes leads to undesirable quality variations in meat [4]. This requires the development of new methods to extend the shelf life and improve the safety of meat [5]. In recent years, much attention has been focused on extracts from herbs and spices [6], which are rich in phenolic compounds [7]. Phenolic extracts have been used to improve sensory characteristics such as taste and flavour [8], as well as to increase the shelf

–life of food [9] by decreasing fatty acid oxidation [10] and microbial growth [11]. Phenolic compounds decrease the development of antibiotic resistance [12, 13]. However, phenolic compounds may interact with medications [14], yet many people do not consider them [15]. *L. nobilis* leaves and fruit have traditional uses in the Mediterranean region for flavour and popular medicine for treating viral infections, cough, rheumatism, diarrhoea, and other health conditions [16]. Many studies have reported on the positive effects of phenolic compounds, including those in *L. nobilis* leaf extracts, such as antimicrobial [17], anti-fungal, antioxidant [18], anti-inflammatory [19], anti-diabetic effects [20], and anticoagulant [21] and anticancer effect [22], where they have various applications in foods and pharmaceutical industries [23]. *L. nobilis* leaves are a rich source of many different phenolic compounds such as flavonoids, phenolic acids, tannins, and lignins [24]. Antimicrobial agents, such as sodium benzoate and benzoic acid, are used as preservatives in the food industry. However, they have harmful effects [25]; for example, the use of sulfite compounds may cause some side effects like headaches and allergies. Another example is the use of benzoate as an antimicrobial agent, which has been suspected to cause allergies, asthma, and skin rashes. Because of the undesirable effects, consumers prefer to eat food free of preservatives when possible, or food containing natural preservatives [26].

Our study aims to evaluate the ability of *L. nobilis* extract to inhibit bacterial growth and thereby improve the shelf life of chicken meat.

## II. MATERIAL AND METHODS

### A. Instrument

Water bath ultrasonic (k &H industries) spectrophotometer (jasco v-530 uv), analytical balance (RADWAG, AS 220/C/2), refrigerator (Hi life), Incubator (JRAD), and autoclave (NUVE stream art model 40L)

### B. Chemicals

Folin–Ciocalteu reagent was purchased from Sigma-Aldrich, Switzerland. Sodium carbonate was obtained from BDH, England. Gallic acid was purchased from Biotech LTD, India. Nutrient agar (NA) was purchased from TM Media. India.

### C. Samples

Fresh *L. nobilis* leaves harvested from the Homs region of Syria were washed with distilled water, dried at room temperature in the dark, then ground in a blender and stored in sterile plastic containers at room temperature.

Chicken meat samples were collected from a local supermarket in Homs,



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# Investigation of the Effect of *Origanum Majorana* on Polycystic Ovary Syndrome Patients

placed in sterile plastic bags, and stored in an icebox at  $4 \pm 2$  °C during transportation for microbiological testing.

## D. Aqueous Extracts

1g of *L. nobilis* leaves powder was added to 20 ml of distilled water. The mixture was then placed in an ultrasonic bath at 50 °C for 45 minutes and then filtered through Whatman paper. The extracts were lyophilised, and the residue was stored at -20 °C in dark glass containers until analysis [27].

## E. Total Phenolic Content

The total phenolic content of the dry extract was determined using the Folin-Ciocalteu method, where 10 mg of dry extract was dissolved in 10 ml of sterile distilled water (1 mg/ml). Then 0.1ml was taken and mixed well with 2 ml of freshly prepared 2% w/v sodium carbonate; after 5 minutes, 0.1ml of 1:1-diluted Folin-Ciocalteu reagent was added. The reaction mixture was allowed to stand at room temperature for 30 minutes. The absorbance was measured with a spectrophotometer at 750 nm. The determination was performed three times. Distilled water served as a blank, and Gallic acid was used as a standard for phenolic compounds [28]. Total phenolic contents were calculated from standard curves of gallic acid solutions in concentrations between (0.1-0.6 g/l) ( $y=1.5945 X - 0.0547$ ,  $R^2=0.9991$ ). The results were expressed as gallic acid equivalents (GAE) in 1 g of *L. nobilis* leaves [29].

## F. Preparation of *L. Nobilis* Extracts and Doxycycline for the Treatment of Meat

*L. nobilis* extract was prepared at a concentration of 900 ppm by dissolving 446 mg of lyophilised extract in 50 mL of sterile distilled water. Then two dilutions (300 ppm and 100 ppm) were prepared by taking 8.3 ml and 2.7 ml, respectively, and diluting each to 25 ml. Doxycycline solution (100 ppm) was prepared by dissolving the contents of one capsule 100 mg in 1000 mL sterile distilled water.

## G. Chicken Sample Preparation

The chicken meat sample was cut into small pieces ( $2 \pm 1$  g) and divided into five groups. The first group, without any treatment, served as the negative control; the second group was treated with Doxycycline (100 ppm). The other three groups were each dipped for 5 min in a solution containing 100, 300, or 900 ppm of *L. nobilis* extracts. Each group was maintained in sterile plastic containers and stored at 4 °C in the refrigerator for 15 days. Bacterial counts were monitored after 1, 7, and 14 days following dipping the chicken samples in the extracts. One small piece from each group was placed in an empty sterile plastic container and weighed; then, 20 ml of physiological saline was added, and then serial dilutions from  $10^{-1}$  to  $10^{-8}$  were performed by the addition of 1ml from the previous solution into 9 ml (0.9NaCl) physiological serum [30]. To determine the total bacterial count, 50  $\mu$ l of the final dilution was spread onto nutrient agar. Then, bacteria were plated on nutrient agar using a loop and incubated at 37 °C for 24 hours [31]. The visible colonies formed on the agar were counted and calculated as the logarithm of colony-forming units per gram ( $\log_{10}$ CFU/g) of samples.

## H. Statistical Analysis

All results are presented as mean  $\pm$  standard deviation. The differences between negative control (chicken samples without treatment), positive control (chicken treated with Doxycycline), and chicken treated with phenolic extract were tested with the student's t-test. Differences were considered to be significant at a p-value  $<0.05$ . All statistical analyses were performed using IBM SPSS Statistics and Microsoft Excel 2013.

## III. RESULTS AND DISCUSSION

### A. Total Phenolic Compounds Determination

Total phenolic contents of the aqueous extract of *L. nobilis* leaves were 24.6035 mg GAE/g. Studies found that the phenolic contents of aqueous *L. nobilis* extract were 42.2 mg GAE/g [32]. Our results are in agreement with other studies, which found that the phenolic compounds in the ethanol *L. nobilis* extract were 25.70 mg GAE/g, whereas the flavonoids were 12.11 mg GAE/g. *L. nobilis* also contains caffeic acid, apigenin, epicatechins and others [33].

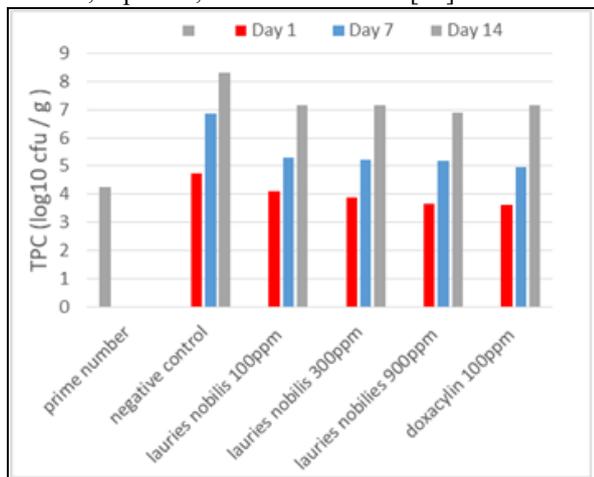
The differences in total phenolic content between our results and those in the literature can be attributed to several factors, including the preparation conditions of extracts [34, 35], the part of the plant used [36], the stage of development at the time of harvest [37], and the type of solvent used [38].

### B. Total Plate Count (TPC) of Treated Chicken Meat Samples

The TPC of treated chicken meat samples compared to the negative control sample is presented in Fig 1. The effect of *L. nobilis* leaf extract was assessed by monitoring changes in TPC over the storage period. Chicken meat stored at 4 °C was acceptable only on the first and second days. The phenolic *L. nobilis* extract exhibited antibacterial activity on the first day of use, with a concentration of 900 ppm showing the best results. On the first day, TPC decreased from 4.25 Log<sub>10</sub> CfU/g to 3.67 Log<sub>10</sub> CfU/g, for a concentration of 900 ppm, while TPC in the negative control reached 4.74 Log<sub>10</sub> CfU/g. On the seventh day, TPC increased in all treated samples compared to the first day, despite the effect of extracts, where TPC in samples treated with 100 ppm, 300 ppm, and 900 ppm phenolic extracts reached 5.28 Log<sub>10</sub> CfU/g, 5.23 Log<sub>10</sub> CfU/g and 5.167 Log<sub>10</sub> CfU/g, respectively. The bacterial counts of these samples did exceed the safe bacterial limit for fresh chicken meat ( $10^6$  CFU/ g) [39]. In contrast, the negative control count was 6.842 log<sub>10</sub> CFU/g, which is unacceptable for human consumption. On the fourteenth day, only *L. nobilis* extract (900ppm) maintained its effectiveness, where TPC reached 6.904 Log<sub>10</sub> CfU/g, which is less than the permissible limit for stored chicken meat for 15 days  $\{10^7$  CFU/g} [36]. Bacterial growth in the negative control reached 8.315 log<sub>10</sub> CFU/g, which was higher than in chicken samples treated with *L. nobilis* extracts at any concentration ( $p < 0.05$ ); therefore, all extract concentrations had an antibacterial effect. Many studies have reported. the antibacterial activity of *L. nobilis* extracts, which enable it is used as a preservative for fresh meat [40]. The results of the current study



are in agreement with another study conducted on chicken treated with methanolic extract of salam leaves in concentrations 0.01%-0.1%-1%, leading to a decrease in TPC in all treated samples, where the concentration 1% was the best. The antibacterial effect was related to the presence of flavonoids, saponins, tannins and others [41].



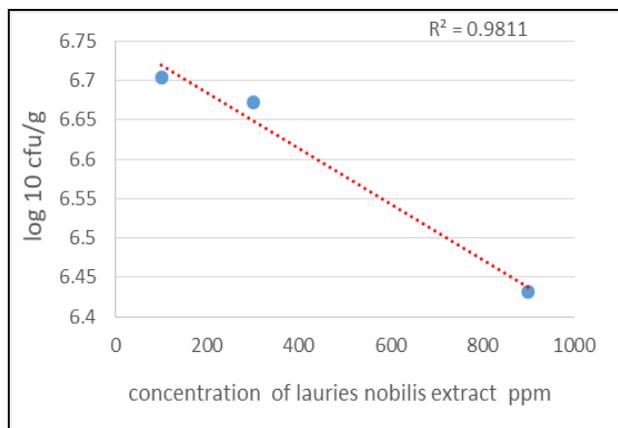
[Fig.1: TPC for Treated Chicken Meat Samples]

### C. Comparison of the Effect of Phenolic L. Nobilis Extract with Doxycycline

Using phenolic extracts (100 and 300 ppm), no difference was noted in the antibacterial effect of L. nobilis extracts compared with Doxycycline (100 ppm) ( $p > 0.05$ ), indicating similar efficacy. The higher concentration of phenolic extract (900ppm) showed a higher antibacterial effect than Doxycycline ( $p < 0.05$ ) as TPC reached on the fourteenth day of extract addition 6.9 Log<sub>10</sub> Cfu/g, versus 7.139 Log<sub>10</sub> Cfu/g after Doxycycline addition.

### D. Study of a Correlation Between Extract Concentration and Antibacterial Efficacy

Fig 2 showed a strong correlation between the concentration of phenolic L. nobilis extract and antibacterial effect ( $R^2=0.9811$ ), where the antibacterial activity increases with increasing concentration of extract. This result is consistent with a study that found that increasing the concentration of salam leaves extract increased the antibacterial effect [42].



[Fig.2: The Correlation Between Extract Concentration and TPC]

## IV. CONCLUSION

The results of our study demonstrate the effectiveness of L. nobilis extract as an antibacterial agent and extend the shelf life of chicken meat during storage at 4 °C for 14 days. This noted activity is related to the phenolic compounds in aqueous L. nobilis extract. The best results for preserving chicken during 14-day storage are achieved with a phenolic extract at 900 ppm.

## DECLARATION STATEMENT

After aggregating input from all authors, I must verify the accuracy of the following information as the article's author.

- **Conflicts of Interest/ Competing Interests:** Based on my understanding, this article has no conflicts of interest.
- **Funding Support:** This article has not been funded by any organizations or agencies. This independence ensures that the research is conducted objectively and free from external influence.
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- **Data Access Statement and Material Availability:** The adequate resources of this article are publicly accessible.
- **Author's Contributions:** The authorship of this article is contributed equally to all participating individuals.

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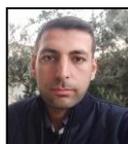


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