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INVESTIGATING THE ACTIVE CHEMICALS IN BAY LEAVES IN SUPPRESSING MICROBES AND IMPROVING THE SENSORY PROPERTIES OF CHILLED CHICKEN MEAT

M. A. Qasim [1] A. A. Al-Azzami [1]* N. T. Khaleel [1] A. A. Yaseen [1]

M. M. Fahad [2]

[1] Department of Food Science, College of Agriculture, University of Anbar.
[2] Center of Desert Studies, University of Anbar.

***Correspondence to:** Ammar Adil Al-Azzami, Department of Food Science, College of Agriculture, University of Anbar, Ramadi. Iraq.
Email: ag.ammar.adil@uoanbar.edu.iq

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Abstract

This study aimed to qualitatively identify the active components in bay leaf extracts and their inhibitory function. While saponins and glycosides were absent, the research did find flavonoids, tannins, alkaloids, phenols, steroids, and terpenes. The investigation showed that the alcoholic extract (ethanol 99.8%) outperformed the aqueous extract in preventing the development and activities of four different types of gram-positive and gram-negative bacteria. The space created by the alcoholic extract was surrounded by a clear zone measuring 18.8, 15.5, 19.48, and 16.8 mm in diameter for the *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Bacillus cereus* culture media, respectively compared to the 10.5, 8.5, 9.4, and 9.1 mm for the aqueous extract. In that order, 1.5% of the alcoholic extract was added at a rate of 0.5, 0.57, and 1.0%. The study's findings also showed that, after 12 days of refrigeration, adding varying amounts of alcoholic extract to chicken meat greatly enhanced its microbiological quality. In the treatment containing 1.5% of the alcoholic extract, the total count of microorganisms, psychrophilic and coliform, reached 7.470, 4.950, and 3.823 cfu\gram, while in the control it was 9.695, 5.873, and 5.386 cfu\gram, respectively. Also, after 12

days of refrigerated storage, the addition of the alcoholic extract enhanced all sensory aspects of the chicken meat as well as its overall level of acceptability.

Keywords: Bay leaf extract, Alcoholic extract, Chicken meat, Refrigerated storage.

التحري عن المركبات الكيميائية الفعالة في ورق الغار وتأثيرها في تثبيط الميكروبات وتحسين الصفات الحسية للحوم الدجاج المبردة

مناف أكرم قاسم [1] عمار عادل العزامي [1]* نور طالب خليل [1] علي أمين ياسين [1]

مصطفى محمد فهد [2]

[1] قسم علوم الأغذية، كلية الزراعة، جامعة الانبار

[2] مركز دراسات الصحراء، جامعة الانبار

***المراسلة الى:** عمار عادل العزامي، قسم علوم الأغذية، كلية الزراعة، جامعة الانبار، الرمادي، العراق.

البريد الالكتروني: ag.ammar.adil@uoanbar.edu.iq

الخلاصة

شملت هذه الدراسة التعرف على المكونات النشطة في مستخلص أوراق الغار ووظيفتها المثبطة نوعياً. ففي حين كانت الصابونينات والكليكوسيدات غائبة، فقد وجدت النتائج مركبات الفلافونويد، التانينات، والقلويدات، الفينولات، والتربينات. كما أظهرت النتائج تفوق المستخلص الكحولي (الإيثانول 99.8٪) على المستخلص المائي في منع نمو ونشاط أربعة أنواع من البكتيريا الموجبة والسالبة لصبغة كرام وذلك باتباع تقنية الحفرة، فقد ظهرت الهالات الشفافة حول هذه الحفر المحتوية على المستخلص بقطر 18.8، 15.5، 19.48، و16.8 ملم في الأوساط الزرعية لبكتريا *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Bacillus cereus* على التوالي. في المقابل، بلغ قطر الهالة الشفافة حول حفر المستخلص المائي 10.5، 8.5، 9.4، و9.1 ملم لنفس الأنواع أعلاه على التوالي. كما أشارت النتائج إلى تحسن الصفات الميكروبية والحسية للحم الدجاج المخزن لمدة 12 يوم على بالتبريد عند إضافة نسب متباينة من المستخلص الكحولي، وإن إضافة نسبة 1.5% من المستخلص تفوقت في القدرة التثبيطية تجاه الميكروبات الملوثة بالمقارنة مع 0.5، 0.57، و1.0% وإنها حسنت كذلك من جودت اللحم المكروبيولوجية والصفات الحسية بعد انتهاء مدة الخزن بالتبريد مما أدى إلى زيادة التقبل العام له. فقد بلغ لوغاريتم العدد الكلي للبكتريا والبكتريا المحبة للبرودة والبكتريا القولونية في المعاملة التي تحتوي على 1.5% من المستخلص الكحولي 7.470، 3.823،4.950 و.ت.م./ غرام في حين كانت هذه الأعداد في إنموذج السيطرة 9.695، 5.873، 5.386 و.ت.م./ غرام على التوالي.

كلمات مفتاحية: مستخلص ورق الغار، الاستخلاص الكحولي، لحم الدجاج، الخزن المبرد.

Introduction

Bay leaf, known as bay laurel, is derived from *Laurus nobilis L*., a little evergreen tree, and is used in a wide variety of recipes. It is classified under the laurel family (Lauraceae) (11). The lands of the Mediterranean basin and the southern and central United States, Europe, the Middle East, and Asia are the original homelands of this plant (5). Bay leaves were used throughout ancient times for decorations and painting to symbolize prosperity, victory, and fame, as well as a remedy and protection against diseases. Some history books mention that they were planted near or in homes as protection from thunderstorms (15), while many aromatic herbs and bay leaves were used as flavor compounds (12). Today, fresh or dried bay leaves are widely used to flavor culinary preparations, while its essential oils are important in the perfume industry. It has also been used in classic medicine due to its various pharmacological activities, including antimicrobial, antioxidant as well as anticancer, insecticidal, and antifungal properties (10).

Fresh bay leaves contain 50% water (6), while dry ones contain only 5-10% water, 65% carbohydrates, 8-11% protein, 5% fat, and 4% ash. They also have about 140 different types of flavor compounds (17). The leaves also contain sugars like fructose, sucrose, glucose, and trehalose, and polysaccharides such as alginate, fucoidan, and laminaran, as well as organic acids like oxalic, malic, and ascorbic acids. In addition, it contains several fatty acids, like palmitic acid and linoleic acid (7). Bay leaves are characterized by their limited, bitter texture as they contain many essential oils and tannins, citric acid, eugenol, flavonoids, carbohydrates, alkaloids, steroids, and triterpenoids. In addition, bay leaves have been found to contain tocopherols, namely α, β, γ, and have the highest concentrations of α and γ. tocopherols, while β-tocopherol is the most prevalent in the seeds. The least abundant component in bay organs namely leaves and seeds, is β-tocopherol (18). The amounts of essential oils in them range from 0.3% to 3%, and because of their low fat and calorie contents, they are considered major sources of vitamin A, various minerals, and fiber (19). Due to their high antioxidant levels they are used to treat stomach ailments and for clearing up mucus in the lungs. They contribute to the prevention and treatment of colds and sore throats (9) and to treat headaches and gastrointestinal problems. These substances exhibit therapeutic and pain-relieving properties by having anti-diarrheal, anti-diabetic, and anti-inflammatory properties (20). Bay leaves exhibit inhibitory properties against several bacterial strains, particularly gram-positive bacteria. Additionally, their extracts have shown the capacity to diminish the activity of molds (2). Adding bay leaves to meat is also thought to enhance the taste of meat. The aim of this study was to determine the effect of adding bay leaf extract to minced chicken meat, its ability to preserve the meat by inhibiting microorganisms, and minimizing alterations to the meat during refrigeration.

Materials and Methods

Preparation of the aqueous extract: Bay leaves collected from local markets in Ramadi city were sent to the laboratory and ground to a fine powder using an electric grinder and then stored in opaque glass bottles until use. The aqueous extract was

prepared by adding 400 ml of distilled water to 20 g of bay leaf powder with continuous stirring for 24 hours. After that, the mixture was filtered and the supernatant was concentrated using a rotary evaporator at a temperature of 40°C. The concentrated supernatant was dried at the same temperature for 3 days to obtain the dry extract powder. The powder was then stored in a refrigerator until use (16). Meanwhile, the alcoholic extract was prepared by adding 500 ml of 99.8% ethyl alcohol to 200 grams of dried powder using the same extraction steps described above (13).

Determining the inhibitory effectiveness of extracts: Four bacteria, two gram-positive and two gram-negative, i.e., *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Bacillus cereus*, were used to determine the inhibitory activity of bay leaf extracts. The purity, viability, and activity of the bacteria were confirmed through phenotypic and biochemical analysis. They were prepared twice in a medium at the minimum growth temperature for 24 hours. The Well Diffusion Assay process was used to test the antagonism ability of the aqueous and alcoholic extracts and to identify which was more dynamic. One ml of activated bacterial suspension was spread in Muller Hinton Agar, after which three holes were made with a cork drill. Using a micropipette, 50 µL of the extract was added at 50 and 100% concentrations in each hole, while the control hole was filled with distilled water. All plates were placed in the incubator at 37°C for 24 hours, and the inhibitory ability of the extracts was determined by estimating the diameter of the clear zones that formed around the holes (23).

Qualitative detection of active compounds in bay leaf extract: Qualitative detection of flavonoids, tannins, saponins, alkaloids, phenols, and terpenes was carried out according to the method described by (13), while that of glycosides and steroids was based on the procedure described by (8).

Preparation of the chicken meat: This study was conducted at the central laboratory of the College of Agriculture, Al-Anbar University. Twelve broiler birds were slaughtered, their carcasses cut, the skin and bones removed, and the meat diced using an electric grinder to a diameter of 8 mm. The meat was divided into five 1-kg treatment groups and the extracts added to the meat as follows: control treatment (T1) no extract, second treatment (T2) 0.5% extract, third treatment (T3) 0.75% extract, fourth treatment (T4) 1% extract, and the fifth treatment (T5) 1.5% extract. To ensure uniform extract distribution the meat was re-diced. It was then prepared again and the samples placed in cork containers and refrigerated at 4°C. Tests were conducted at days 0, 3, 6, 9, and 12.

Microbial tests:

Total number of bacteria: The pour-plate method described by (4) was followed using Nutrient agar. Ten grams were taken from each treatment of stored meat and mixed with 90 ml of sterile Peptone water. A series of dilutions was prepared, and 1 ml of each dilution was withdrawn and placed in a sterile Petri dish, and the culture medium poured over it. The dishes were incubated in a JSR incubator (Korean-made, model JSGI-50T) at 37°C for 24-48 hours. The number of growing colonies was determined using a colony counter. The same steps were followed in counting

bacteria during the storage time, taking into account the use of a control dish for each treatment.

Psychrophilic bacteria: The number of psychrophilic bacteria in the meat samples was determined using the method described by (3). One ml of the series of dilutions was transferred to a sterile Petri dish and Nutrient Agar added. The dishes were then incubated at 7°C for 5-7 days.

Coliform Bacteria Count: Coliform Bacteria Count was determined according to the method mentioned by (4) using the Mac-Conkey Agar medium. The series of dilutions method and the plates were incubated at 37°C for 24-48 hours, and the bacteria colonies growing in the dishes were counted using the colony counter.

Sensory evaluation: Sensory evaluation scores were estimated for the characteristics of color and flavor, texture, juiciness, and general acceptability of samples of minced chicken meat prepared by cooking in an oven at 165°C until the internal temperature of the meat reached 70°C. Sensory evaluation scores were given by a panel of 10 graders using a scale from 0 to 10 according to the sensory evaluation form mentioned by (24).

Results and Discussion

The results in Table 1 show that the alcoholic bay leaf extract was superior to the aqueous extract in its inhibitory ability against the four types of bacteria, and this ability was proportional to the increase in the concentration of the extract. The 50% aqueous extract concentration failed to inhibit any of the bacterial species, while the same amount for the alcoholic extract produced clear zones with diameters of 17.2, 12.4, 16.2, and 11.2 mm in the Petri dishes containing *Satph. aureus*, *B. cereus*, *E. coli* and *Sal. typhi*, respectively. Also, the clear-zones diameters in the dishes containing 100% concentrations of alcoholic extract were 19.4, 16.8, 18.8, and 15.5 mm for *B. aureus*, *B. cereus*, *E. coli*, and *Sal. typhi* respectively.

Table 1: Effectiveness of aqueous and alcoholic bay leaf extracts in inhibiting different types of bacteria.

Type of Bacteria	Type of Extract	Diameters of the Clear Zones (mm)	
		50%	100%
Staphylococcus aureus	Alcoholic	17.2	19.4
	Aqueous	0	10.5
Bacillus cereus	Alcoholic	12.4	16.8
	Aqueous	0	8.5
Escherichia coli	Alcoholic	16.2	18.8
	Aqueous	0	9.4
Salmonella typhi	Alcoholic	11.2	15.5
	Aqueous	0	9.1

Table 2 shows the results of the qualitative investigation of the active compounds in the alcoholic extract of bay leaves. The alcoholic extract was used to investigate the effective compounds after obtaining the results of its inhibitory effectiveness and confirming its superiority over the aqueous extract.

Table 2: Active compounds in the alcoholic extract of bay leaves.

Active Compounds	Investigation Results*
Flavonoids	+ve
Tannins	+ve
Saponics	-ve
Alkaloids	+ve
Glycosides	-ve
Phenols	+ve
Steroids	+ve
Terpenes	+ve

***+ve = positive; -ve = negative.**

The table shows that the extract contained flavonoids, tannins, alkaloids, phenols, steroids, and terpenes but lacked saponins and glycosides. Flavonoids are considered effective compounds having antioxidant and antibacterial properties that can suppress the growth and activity of many microorganisms (22). They prevent the growth of bacteria and fungi thus enabling their use for preserving food (14). Many studies prove the role of natural extracts and their safe use in preserving meat products from chemical and biological deterioration, thereby improving their microbial and sensory characteristics as well as promoting consumer acceptance (1).

As seen in Table 3, there is no major difference in total microbe count in the chicken meat treated with different amounts of alcoholic bay leaf extracts at the beginning of the experiment. However, the T1 group showed significant superiority ($p<0.05$) on the third day of storage with numbers reaching 5.645 cfu\gram compared to 4.945, 4.928, and 4.922 cfu\gram for T3, T4, and T5, respectively. The T5 group recorded the largest decrease ($p<0.05$) in microorganism numbers after 6 and 9 days storage at 5.495 and 6.409 cfu\gram, respectively. After 12 days of storage, T4 and T5 recorded the largest decrease ($p<0.05$) with the number of microorganisms registering 7.476 and 7.470 cfu\gram, respectively. The decrease for the treatments containing the alcoholic extract compared to the control is due to the presence of active compounds in the bay leaf extract, which worked to suppress the growth and activity of microbes during the storage period. This negatively affected the total number of microbes thereby improving the preservation capacity for refrigerated chicken meat.

Table 3: Effect of adding bay leaf extracts on total bacteria count (cfu\gram) in chilled chicken meat.

Treatment	Storage Duration				
	0 days	After 3 days	After 6 days	After 9 days	After 12 days
T1	4.671	5.645 **a**	7.751 **a**	8.845 **a**	9.695 **a**
T2	4.690	5.351 **b**	6.000 **b**	7.520 **b**	8.218 **b**
T3	4.691	4.945 **c**	5.987 **b**	7.015 **c**	7.830 **c**
T4	4.695	4.928 **c**	5.805 **b**	6.622 **d**	7.476 **d**
T5	4.699	4.922 **c**	5.495 **c**	6.409 **e**	7.470 **d**
Prob.	NS**	< 0001	< 0001	< 0001	< 0001
Total mean	4.689	5.158	6.207	7.282	8.138
sem	0.014	0.081	0.213	0.232	0.222

** NS: non-significant. Different subscripts (a, b, c) in the same columns show significant differences at probability value (P≤0.05).

Table 4 shows the effect of including bay leaf alcoholic extracts on the number of psychrophilic microorganisms in chicken meat during refrigeration. There were no statistically significant variations ($p<0.05$) in the numbers at the beginning of the experiment or across all treatments. Nevertheless, large disparities are seen in the numbers following three days of storage, with the control group outperforming the other treatments with the logarithm of the psychrophilic numbers at 4.935 cfu\gram for the control group, while T5 had the lowest value at 4.752 cfu\gram and T4 recorded 4.791 cfu\gram.

Table 4: Effect of adding bay leaf extracts on the psychrophilic organisms of minced chicken meat.

Treatment	Storage Duration				
	0 days	After 3 days	After 6 days	After 9 days	After 12 days
T1	3.817	4.935 a	5.096 a	5.531 a	5.873 a
T2	3.811	4.898 b	4.967 b	5.306 b	5.877 a
T3	3.794	4.861 c	4.927 b	5.016 c	5.336 b
T4	3.816	4.791 d	4.844 c	4.918 d	4.972 c
T5	3.810	4.752 e	4.824 c	4.856 e	4.950 c
Prob.	NS**	< 0001	< 0001	< 0001	< 0001
Total mean	3.809	4.847	4.932	5.125	5.401
sem	0.008	0.018	0.026	0.068	0.110

** NS: non-significant. Different subscripts (a, b, c) in the same columns show significant differences at probability value (P≤0.05).

T5 and T4 recorded the most significant decrease in psychrophilic bacteria after 6 days at 4.824 and 4.844 cfu\gram, respectively. At the end of the 12-day storage

period, the control was significantly superior to the other treatments at 5.873 cfu\gram, while T3, T4, and T5 recorded 5.336, 4,972, and 4.950 cfu\gram, respectively. In conclusion, bay leaf extracts effectively suppress psychrophilic bacteria activity, negatively affecting their final numbers. This may be due to the active compounds that affect the walls of the living cell.

Table 5 shows the effect of adding bay leaf extract on the number of coliforms in chicken meat during refrigeration, as a significant ($p<0.05$) increase can be observed in their numbers in the control compared to the other treatments and for all except the first storage period. While there was a significant decrease ($p<0.05$) in coliform in treatments T3, T4, and T5 after three days of refrigeration, the logarithms of the bacterial numbers recorded 3.582, 3.525, and 3.512 cfu\gram, respectively, compared to 3.783 cfu\gram for T1. A significant decrease was also observed ($p<0.05$) for T3, T4, and T5 at the end of 12-days storage at 4.039, 3.912, and 3.823 cfu\gram, respectively, compared to the control at 5.386 cfu\gram.

Table 5: Effect of adding bay leaf extracts on the coliform numbers (cfu\gram) of cold-stored chicken meat.

Treatment	Storage Duration				
	0 days	After 3 days	After 6 days	After 9 days	After 12 days
T1	3.297	3.783 a	4.103 a	4.782 a	5.386 a
T2	3.294	3.595 b	3.842 b	4.003 b	4.615 b
T3	3.296	3.582 bc	3.793 bc	3.895 bc	4.039 c
T4	3.293	3.525 bc	3.747 c	3.842 c	3.912 d
T5	3.294	3.512 c	3.730 c	3.757 c	3.823 d
Prob.	N S**	< 0001	< 0001	< 0001	< 0001
Total mean	3.295	3.599	3.843	4.056	4.355
sem	0.010	0.027	0.037	0.100	0.156

** NS: non-significant. Different subscripts (a, b, c) in the same columns show significant differences at probability value ($P\leq0.05$).

Table 6 shows the effect of adding bay leaves extracts on the degree of consumer acceptance of stored chicken meat at the end of the refrigerated storage periods. Significant differences ($P\leq0.05$) are seen in the experimental compared to the control treatments, which recorded a clear decline in all traits. T4 was significantly superior ($P\leq0.05$) in terms of color and flavor and not significantly different from treatment T3 for these traits, while treatments T3, T4, and T5 were markedly superior ($P\leq0.05$) in the traits of texture and juiciness. As for overall acceptability, T3 and T4 were significantly superior ($P\leq0.05$). The results clearly show that adding extracts to chilled minced chicken meat increased consumer acceptance. This is due to the product's preservation during storage as the effective compounds of the alcoholic extract in the bay leaves work to preserve the characteristics of the meat (21). Many studies on the role of natural extracts in this aspect confirm these findings.

Table 6: Effect of adding bay leaf extracts on the sensory evaluation of chilled chicken meat.

Treatment	Scores				
	Color	Flavor	Texture	Juiciness	Overall Acceptability
T1	6.00 d	5.80 d	6.20 b	5.80 c	6.00 c
T2	6.80 c	6.80 c	6.80 b	7.00 b	7.00 b
T3	7.80 ab	7.60 ab	7.90 a	8.00 a	7.80 a
T4	8.10 a	8.10 a	8.10 a	8.40 a	8.10 a
T5	7.20 bc	7.00 bc	8.20 a	8.40 a	7.20 b
prob	<.0001	<.0001	<.0005	<.0001	<.0001
Total mean	7.18	7.06	7.44	7.52	7.22
sem	0.175	0.183	0.206	0.238	0.168

** NS: non-significant. Different subscripts (a, b, c) in the same columns show significant differences at probability value (P≤0.05).

Conclusions

This research aimed to qualitatively identify the active components and inhibitory effects of bay leaf extracts. Saponins and glycosides were lacking, but flavonoids, tannins, alkaloids, phenols, steroids, and terpenes were found. Ethanol 99.8% exceeded the aqueous extract, preventing 4 gram-positive and gram-negative bacteria from growing and reproducing. The study found that adding varied volumes of alcoholic extract to chicken meat improved its microbiological quality after chilling. Also, adding alcoholic extract enhanced the sensory features of chilled chicken meat and improved its acceptability after 12 days of refrigerated storage.

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All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

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