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Application of plant extracts as bio preservatives against *Listeria monocytogenes* and antioxidant in meat patties

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ABSTRACT. This study aimed to investigate the antioxidant and antibacterial properties of plant extracts derived from kiwi peels (*Actinidia deliciosa*), beet peels (*Beta vulgaris* L.), eggplant peels (*Solanum melongena*), and red cabbage leaves *(Brassica oleracea* L. var). Total phenols, flavonoids, free radical scavenging activity (DPPH), reducing power, and ferrous ion chelating capacity were evaluated. Beet peel extract showed the highest phenolic and flavonoid contents and exhibited the strongest DPPH scavenging activity, reaching 85% at 100 mg mL^{-1}, as well as the most effective iron chelation, while eggplant peel showed the lowest activity. To assess preservative potential, kiwi and red cabbage extracts (0.5, 1, and 1.5%) were incorporated into minced meat patties inoculated with *Listeria monocytogenes* or stored as non-inoculated controls at 4 ± 1°C for 12 days. Microbiological analysis revealed significant reductions in total aerobic bacteria and *L. monocytogenes* counts, with kiwi peel extract showing the strongest inhibitory effect. Furthermore, treated patties demonstrated lower peroxide and free fatty acid values compared to controls (2.8 and 1.27 mEq kg^{-1}, respectively, on day 12). These findings highlight the potential of selected plant extracts, particularly kiwi peels, as natural antioxidants and bio-preservatives in meat products.

Keyword: Natural antimicrobials*;* phenolic compounds; green preservation; lipid oxidation; food safety; meat quality.

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Introduction

Meat is an important part of the human diet since it offers the majority of the needed nutrients for humans, as well as energy and easy-to-digest proteins that are high in vitamins and minerals (Ashar et al*.,* 2022). Meat proteins are highly regarded due to the abundance of essential amino acids, which can enhance the nutritional value of meat-based products. *Listeria monocytogenes* is one of the most major causes of meat spoiling throughout preservation by cooling, freezing, and salting, since they are microorganisms that are resistant to cold, as they thrive at temperatures (2-4) C, and are also resistant to salinity by 18-20% (Wang et al*,* 2024 Al-Hamdani & Al-Noor (2024), A significant moisture content and a neutral pH are optimal for the growth and reproduction of the majority of pathogenic bacteria that cause spoiling (Mohammad and Andyousif, 2022, Andriyanov et al, 2021). They are among the most significant variables resulting in illnesses caused by food, as these bacteria are transmitted to the body of humans and cause recurrent miscarriage in pregnant women, meningitis, and blood cell breakdown in newborns, and the people the majority at risk of contracting listeria are those with immunodeficiency, such as cancer and AIDS patients (Moura et al*.,* 2024; Saleh et al*.,* 2024). Bacteria have become more resistant to antibiotics in recent years as they have been used to treat diseases and infections more often. Bacteria are tough to treat, so researchers are looking for alternative sources of antimicrobials, including medicinal plants. Mogana et al. (2020) Plant extracts contain enormous quantities of physiologically active substances, especially polyphenols, which function as antioxidants and antimicrobials, making them the greatest alternative to chemical preservatives. (Efenberger-Szmechtyk et al*.,* 2021). The requirement to obtain natural preservatives for food preservation has grown, as most plant extracts encompass a significant number of phenolic compounds, which are responsible for a variety of antibacterial and antioxidant properties (György et al*.,* 2020). Use natural antioxidants instead of chemical preservatives since they include many beneficial compounds, which involves phenolic substances and flavonoids, that are effective in scavenging radicals that are harmful as well as other health advantages (Munekata et al*.,* 2020; Sood et al., 2020) The study aimed to The current study aims to evaluate the activity of some plant extracts as natural antimicrobials, especially against the bacteria *L. monocytogenes,* and evaluate their effectiveness in prolonging the shelf life of minced meat patties stored refrigerated for a period at a temperature of 4°C.

Materials and methods

Plant materials

The plant specimens were purchased from local markets in Basra, southern Iraq a city in Western Asia characterized by a hot desert climate with long, dry summers and mild winters, and dried under controlled conditions at 40°C for a period not exceeding 48 ± 2 hours, depending on the type of plant. Then they were ground with an electric grinder and kept in polyethylene bags and preserved by freezing until use.

Preparation of plant extracts

All The plants employed in this research were purchased from local markets in the city of Basra, including (kiwi *Actinidia deliciosa*, eggplant *Solanum melongena*, red cabbage *Brassica oleracea L.var*., and beetroot *Beta vulgaris L.*) targeted plants dried at 40°C for 48 ± 2 hours, depending on the type of plant, before being ground with an electric grinder. Then, 100 g of each plant's powder was weighed, and 500 mL of 98% ethyl alcohol, mixed well and left for an hour at a laboratory temperature of 25°C. The solution was then filtered using Whatman No. 1 filter papers. Meanwhile Collected the filtrate and concentrated with a rotary evaporator at a temperature of 40°C to extract the solvent (Kamal et al., 2019). The filtrate was then dried at laboratory temperature and placed in tightly sealed containers until used.

Determination of total phenolic contents

The total phenolics of alcoholic and aqueous plant extracts were estimated using the Ciocalteu-Folin method described by Karadeniz et al. (2024) with some modifications. The method involved dissolving 0.5 g of plant extracts in 5 mL of distilled water, then taking 1 mL of the dissolved plant extracts and adding 1 mL of Ciocalteu-Folin reagent and mixing well. After 5 minutes, 2 mL of Na_2CO_3 (20%) was added, and the mixture was placed in a dark area for an hour with occasional shaking, before the intensity of absorption was measured at a wavelength of 760 nm.

Determination of total flavonoids contents

Equivalent flavonoids were calculated using the method described in (Viera et al., 2017) using Rutin as a standard at concentrations (10-120 mg mL^{-1}), in which 0.5 mL of plant extracts were deposited in a test tube followed by 150 microliters of (5%) $NaNO_2$ and after 5 min 150 µL of aluminum chloride$AlCI_3$ addition. After 6 min, 1 mL of 1 M sodium hydroxide (NaOH) was added. After that, 1.2 mL of distilled water was added and the absorbance was measured at 510 nm.

Determination of Antioxidant Activity of plant extract using DPPH

Antioxidant activity were carried out in the manner suggested by Brahma and Baruah (2023) The procedure consisted for preparing concentrations of the phenolic plant extracts that ranged from 10 t0 100 mg mL^{-1}. Then 1 mL of each concentration were mixed with 0.01% DPPH and then allowing the mixture incubated in the dark for 30 min. Following that, absorbance was measured at 517 nm, the control sample was prepared in the same manner as the test sample, except that methanol was used instead of the test sample. The synthetic antioxidant (BHT) was applied at the same concentration for comparison, DPPH activity was calculated using Equation 1:

$$DPPH\ scavenging\ activity\ (\%) = [\frac{(control\ absorbance - sample\ absorbance)}{control\ absorbance}] \times 100 \quad (1)$$

Measurement of reducing power

Applying the procedure of Sathisha et al. (2011), 2.5 mL of plant extracts at a concentration of 10to100 mg mL^{-1} made with distilled water, were mixed with 2.5 mL of phosphate buffer an approach 200 mM, pH 6.6, and 2.5 mL of potassium ferricyanide solution. (1%) The mixture was incubated at 50°C for 20 minutes. 2.5 mL of Trichloroacetic acid (1%) was added. The mixture was centrifuged at 2000 rpm for ten minutes. The solution's upper layer was separated, and 5 mL of purified water and 1 mL of ferric chloride (0.1%) were added. Absorption was measured at 700 nm. The control sample was created by adding all of the previous components except 2.5 mL of ethanol instead of extracts from plants, and ascorbic acid was utilized for comparison.

Chelating ability of ferrous ion

The potential ability of plant extracts to bind iron ions was measured using the method given in(Patel, 2013) which included mixing 0.4 mL of plant extracts at concentrations ranging from 1 to 5 mg mL^{-1} with 0.4 mL of 2 mM ferrous chloride and 0.4 mL of 5 mM 8-hydroxyquinoline (prepared with 98% ethanol). Incubate the mixture for 10 minutes at room temperature in a dark area. Measure absorbance at wavelength 562nm. To provide a comparison, the ferrous ion binding ability of Ethylene Diamine Tetra acetic Acid Disodium Salt (EDTA-2Na) was calculated in the same manner. The sample used for control was made in the same manner as described above, with the exception of the addition of botanical extracts. The capacity of the plant extracts to attach to ferrous ions was estimated using the following Equation 2:

$$Chelating\ ability\ of\ ferrous\ ion\ (\%)\ = 1 - [\frac{(sample\ absorbance)}{control\ absorbance}] \times 100 \quad (2)$$

Growth control of *listeria monocytogenes* by plant extracts in artificially contaminated meat patties

Meat discs weighing 8 kg were prepared using an electric machine at a rate of 50 grams per treatment, then divided into eight treatments, after which plant extracts were added to the meat discs in different concentrations as shown in the Table 1.

Table 1. Inoculation trials in meat patties.

No.	Treatment
T1	Bacteria free
T2	Inoculated with bacteria 108
T3	Kiwi 0.5%
T4	Kiwi 1%
T5	Kiwi 1.5%
T6	Red cabbage 0.5%
T7	Red cabbage 1%
T8	Red cabbage 1.5%

All meat patty treatment samples were refrigerated at 4 ± 1°C for 10 days and then monitored for changes in chemical indicators and microbial activity.

Studying the changes occurring in the microorganisms of cold-stored meat patties

Estimating the total number of aerobic bacteria

The cultivation medium, Nutrient Agar, was prepared according to the manufacturer's recommendations (Himedia, India), sterilized, and poured into Petri dishes. It was then possible to calculate the total quantity of aerobic bacteria using the technique of spreading on the dishes as mentioned in the method of Bonos et al. (2022), who prepared a series of decimal dilutions using a peptone water solution as illustrated below (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}). 0.1 mL of the dilutions 4-10 and 5-10 were collected and published on The Petri dish was set with an L-shaped glass rod for 10 minutes before being incubated upside down at 37°C for 18-24 hours. Colonies on the dish were then counted.

Determanation of *Listeria monocytogenes* bacteria

The colonies *of Listeria monocytogenes* were identified using the spreading method, which involved spreading 0.1 mL of dilutions 1-10 and -210 onto the selective medium PALCAM Agar. The plates were incubated at 37°C for 18-24 hours, and the colonies forming on the plate were counted (Benyagoub et al., 2024).

Chemical property tests

Chemical characteristics were estimated using typical methodologies given in existing literature. provided a method for determining the peroxide value (PV) in meat patties, whereas the percentage free fatty acids FFAs was measured using the approach presented in The Association of Official Analysis Chemists [AOAC] (1990).

Statistical analysis

Statistical analyzes for data were designed using a completely randomized design (CRD). a two-factor experimental experiment, and a three-factor factorial experiment were employed. For the analysis of variance

(ANOVA) on the experimental data, the data was analyzed statistically using the SPSS software version 26 in 2019. These factors were examined using the Least Significant Difference (L.S.D.) which was attributed to the 0.05 level (Genstat, 2011).

Results and discussion

Total phenolic contents

The total phenolic compound of the plant extracts are shown in Figure 1. The ethanol extract of eggplant peels had 76.25 mg GAE g^{-1} while the extract of red cabbage leaves did not have as many phenols. 79.89 mg GAE g^{-1}, while the total phenol level in kiwi and beet peel extracts increased to 83.13 and 89.21 mg GAE g^{-1}, respectively. The reason for the variance in the percentage of phenolic compounds could be attributed to the type of plant, the molecular weights of the phenolic compounds, and the type of solvent polarity. Used in extraction (Mokaizh et al., 2024; Reshma and Suganthi 2024). This is corroborated by El-Sawah et al. (2024). The percentage of plant extracts rises with increasing solvent polarity. According to Reshma and Suganthi (2024), the ethanol extract included the most phenols in comparison to other solvents, whilst the aqueous extract contained the least quantity of phenolic compounds.

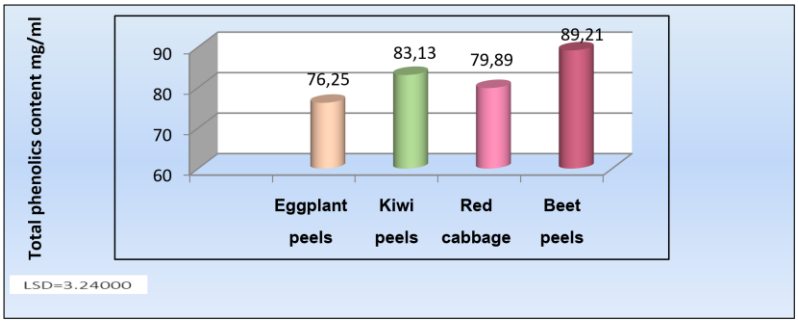

Figure1. Total phenolic contents (TPCs) of the plant extracts (mean ± *SD;n*=3).

Total content of flavonoids

Four plant extracts were evaluated for their flavonoid content: beet peels, red cabbage leaves, kiwi peels, and eggplant peels. The results demonstrated by Figure 2 showed significant differences between the plant extracts from plants, as the smallest amount of flavonoids in the red cabbage leaf extract was 61.92 mg Rutin g^{-1}, whereas beet peels showed the highest concentration, reaching 84.79 mg Rutin g^{-1}, and flavonoids in kiwi peels and eggplant peels were 76.67 mg Rutin g^{-1} and 68.72 mg Rutin g^{-1}, respectively. The amount of yield in aqueous extracts and ethanol extracts differs due to the nature of the chemical composition of the plants and their degree of solubility and the polarity of the solvent and solubility of flavonoids in the solvent (Mikucka et al., 2022).

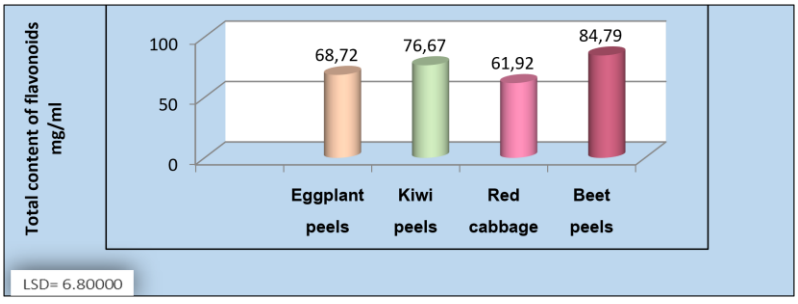

Figure2. Total flavonoids contents (TFCs) of the plant extracts (mean ± *SD; n* = 3).

DPPH radical scavenging activity

Figure 3 clarified the free radical scavenging activities (DPPH) of kiwi peel extracts separately relative to synthetic antioxidant (BHT) at different concentration from (10-100 mg mL^{-1}). The results of the statistical analysis demonstrate the presence of significant differences ($P \leqslant 0.05$), the scavenging activity the DPPH showed significant variations between plant extracts, with the highest percentage in the beet peel extract

reaching 55.75, 65.18, 72.25, 81.46, 85.18, and 88.72% at levels of 10, 20, 40, and 60. and 80 and 100 mg mL^{-1}, respectively, while the smallest percent of DPPH in the eggplant peel extract was 15.88, 20.72, 33.6, 49.27, 58.14, and 67.5% at the same concentrations. DPPH is a chemical molecule used to assess the potency and efficacy of antioxidants. It is a relatively stable free radical with a prominent absorption peak at 517 nm. This radical is commonly used to evaluate the efficacy of antioxidants to scavenge free radicals, as antioxidants interact with the free radical and convert hydrogen molecules into inactive or non-free molecules, reflecting their effectiveness as antioxidants (Liu et al., 2024; Hu et al., 2024). phenolic compounds exhibit a reaction with free radicals by donating electrons, thereby enabling the transformation of free radicals into stable compounds (Hu et al., 2022). Asadi et al. (2024) found that the highest effectiveness of scavenging the free radical DPPH in kiwifruit was 77.26%.

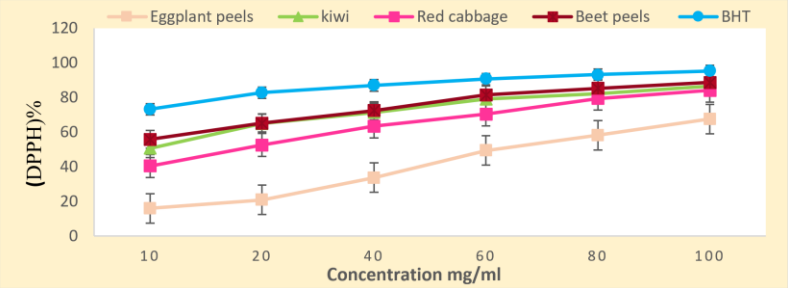

Figure 3. Free radical scavenging ability of plant extracts (mean ± *SD; n* = 3). Bars with different letters differ significantly (p <0 .05).

Reducing power

Reducing power values of plant extracts are shown in Figure 4 and compared with ascorbic acid and. The concentration of 100 mg mL^{-1} had the highest reducing power for eggplant peels, kiwi peels, red cabbage leaves, and peels. Beets 0.433, 0.709, 0.547, 1.114%, while ascorbic acid had an absorbance reading of 2.892 nm. The reducing power of alcoholic plant extracts was determined by examining the relationship between absorbance and concentration. The reducing power was calculated by increasing the absorbance, which is directly proportional to the reduction of the trivalent ferric ion Fe^{+3} and its conversion to the ferrous ion $Fe^{+2,}$ resulting in a potassium ferrocyanide complex under acidic circumstances. Potassium ferricyanide interacts with ferric chloride to produce ferricyanide, with an absorbance peak at 700 nm. The bigger the reductive force, the higher the absorption at 700 nm and the better the antioxidant efficacy (Yu et al. 2024**).**

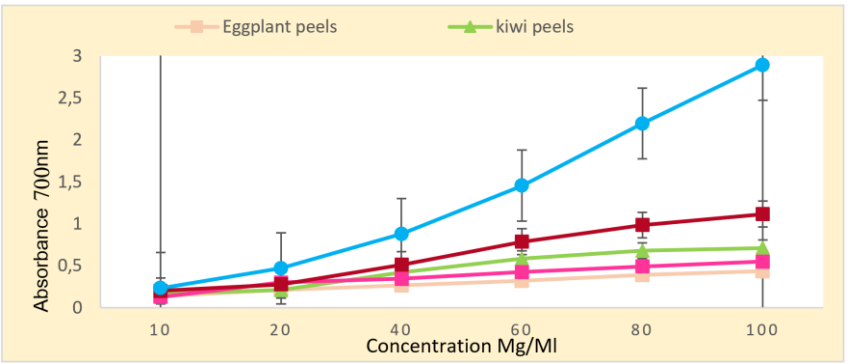

Figure 4. Reducing power of plant extracts (mean ± *SD; n* = 3). Bars with different letters differ significantly (p <0 .05).

Chelating of ferrous ion

Figure 5 shows the ability of plant extracts to bind ferrous ions in comparison with EDTA-2Na using concentrations and ranged (10-100 mg mL^{-1}) as the ability to bind ferrous ions increased with increasing concentration, as the beet extract showed the greatest degree of binding, reaching 81.11%, while the eggplant peel extract showed less effectiveness for binding ferrous ions, reaching 54.48.% We note from the above results that the ferrous ion binding ratios of plant extracts are low in comparison to EDTA -2Na, except for beet extract, whose results are comparable to it, and the antioxidant activity of beet extract is attributed to the content of Betalain, which consists of Betacyanins and Betaxanthins (Banwo et al., 2022). Phenolic compounds contribute to the effectiveness of the binding of the neutralization ion, which means that they

react with iron and form stable complexes with it, and there is a significant relationship between the total content of flavonoids and the ability of compounds to bind ferrous ion (Loizzo et al., 2012). Due to the high oxidative nature of iron, plant antioxidants can decrease the reduction of ferric ion Fe^{+3} to ferrous Fe^{+2}, which is less effective for oxidation. On the other hand, ferrous ion can effectively break down peroxides and hydrogen peroxide H2O2 into free radicals through the Fenton reaction, protecting the biological system from oxidative stress (Paiva et al.,2022; Hassan et al.,2023).

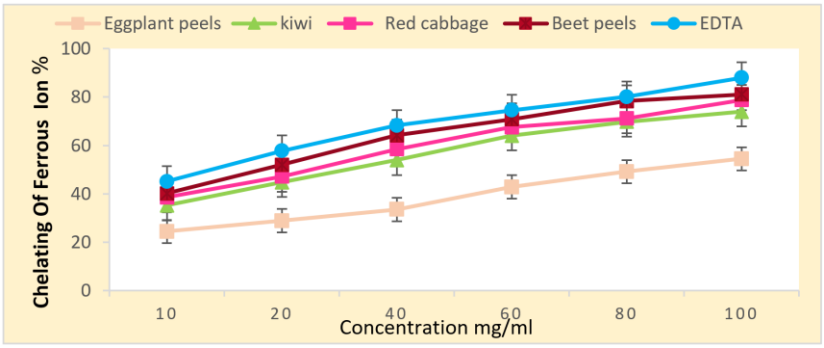

Figure 5. Chelating of ferrous iron plant extracts (mean ± *SD; n* = 3). Bars with different letters differ significantly (p <0 .05).

The effect of adding plant extracts on the total number of aerobic bacteria in minced meat patties stored in cold storage

Figure 6 shows a gradual increase in the averages of the logarithm of the total number of aerobic bacteria for the two treatments: the control sample, which was free of *Listeria monocytogenes*, and the control sample that was inoculated with 108 *Listeria monocytogenes* during Storage stages (0–12) days of cold storage. The aggregate number of aerobic bacteria reached (4.616 and 6.09) CFU g^{-1} on day zero, respectively, as the logarithm of the numbers was (7.708 and 7.881) CFU g^{-1} on day twelve. The statistical analysis revealed significant differences at the probability level (P ⩽ 0.05). The increase in cold-stored meat can be attributed to the growth of certain psychrophilic bacteria that prefer cold temperatures. The addition of red cabbage leaf extract at a concentration of 0.5% was found to slightly inhibit the growth of the bacteria in comparison to the two concentrations (1 and 1.5%), with the highest inhibitory effectiveness observed on day 12 and at a concentration of 1.5%. When the kiwi peel extract was compared to the red cabbage leaf extract at the same concentrations, it had the greatest inhibitory action on aerobic bacteria at all doses, including 0.5%, 1%, and 1.5%. Because of the lower logarithm of the average numbers of all the aerobic bacterial counts, the kiwi peel extract is therefore thought to be superior to the red liana extract. which, at a 1.5% concentration on day 12, reached 2.938 CFU g^{-1}. These findings corroborated those of Hafidh et al. (2011), who found that the red lichen extract was highly effective at suppressing pathogenic microorganisms, including *Salmonella enteric*, *Pseudomonas aeruginosa, Escherichia coli* O157:H7, *Staphylococcus aureus*, and Klebsiella pneumonia. Red cabbage extract's antibacterial properties and abundance in phenolic chemicals and anthocyanins account for its efficacy. Which works to decompose the bacterial cell wall and also disrupts the bacterial cell membranes, especially the internal ones, thus causing cell death (Efenberger-Szmechtyk et al. 2021).

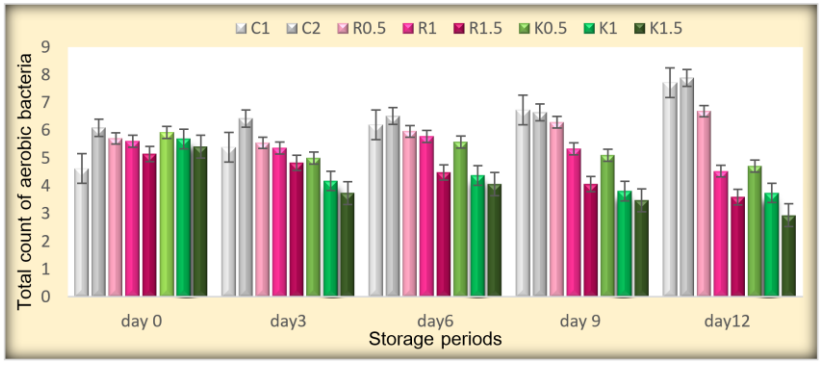

Figure 6. Effect of Alcoholic Plant Extracts on Total Aerobic Bacteria in Minced Meat Patties during Storage (mean ± SD; n = 3). Bars with different letters indicate significant differences (p < 0.05). C1, C2: Controls; R0.5, R1, R1.5: Red lichen extract (0.5–1.5%); K0.5, K1, K1.5: Kiwi extract (0.5–1.5%).3.7. The effect of adding plant extracts on the 3.7. numbers of *Listeria monocytogenes* bacteria.

As a result of the bacteria's ability to grow and reproduce at a temperature of 0 ± 4°C, we observe an increase in the number of *Listeria monocytogenes* in the second control sample that was inoculated with 108 of the bacteria during the cold storage period (Figure 7). In contrast, we observe a gradual decrease in the treatment of the first control sample, which was free of *Listeria monocytogenes* bacteria during the storage period, as the amount of bacteria reached (0, 3, 6, 9, and 12) (2.437, 2.904, 1.178, 1, and 0) CFU g^{-1}, respectively. This is because *Listeria monocytogenes* was not injected into this treatment. Additionally, as the length of cold storage increased, a significant decline was seen in the average logarithms of the number of *Listeria monocytogenes* for all concentrations of the alcoholic extract of kiwi peels. Day 12 at a concentration of 1.5% yielded the lowest logarithmic average of the numbers of *Listeria monocytogenes* since no bacterial growth was seen. The findings are displayed in Figure (7): The alcoholic extract of red cabbage leaves is less effective than the *Listeria monocytogenes* extract from kiwi peels; on day 12, at a concentration of 1.5%, the lowest logarithmic average of the numbers of *Listeria monocytogenes* was 3.019 CFU g^{-1}. These outcomes are comparable to those of Kim et al. (2019) investigation on the antibacterial efficacy of kiwi peel extract on the quality of chicken breast meat tainted with *Listeria monocytogenes* throughout storage for up to nine days at 4 degrees Celsius. Due to the fact that kiwi peel extract is abundant in physiologically active substances such phenolic compounds, Siddique et al. (2021) also discovered that it exhibits antibacterial action against a variety of microorganisms, including *Listeria monocytogenes*.

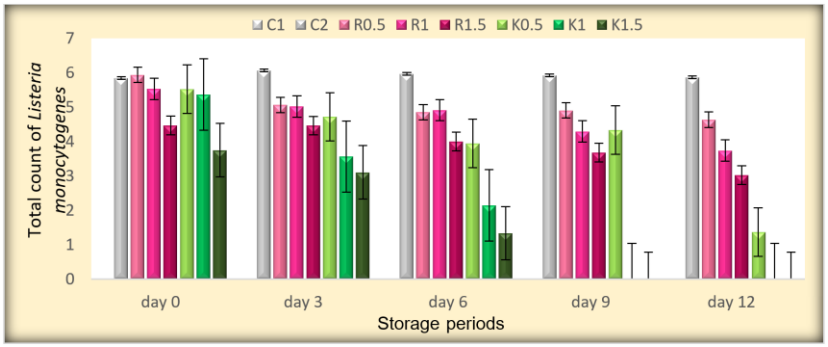

Figure 7. Effect of Plant Extracts on *L. monocytogenes* in Minced Meat Patties during Storage (mean ± SD; n = 3). Bars with different letters indicate significant differences (p < 0.05). C1, C2: Controls; R0.5, R1, R1.5: Red lichen extract (0.5–1.5%); K0.5, K1, K1.5: Kiwi extract (0.5–1.5%).

Chemical properties of plant extracts- incorporated meat patties

The peroxide values PV of minced meat patties that were supplemented with red cabbage leaves and kiwi peels plant extracts and stored by refrigeration at 4 ± 1°C for a period of 0, 3, 6, 9, and 12 days are displayed in Figure 8. The peroxide values were 0.43 mEq O_2 kg^{-1} for all treatments at the start of storage and climbed to 2.8 mEq O_2 kg^{-1} in the control sample by the 12th day of storage, while they were 2.11 and 2.34 mEq O_2 kg^{-1} in meat patties treated with 1.5% concentration of red cabbage and kiwi peel extract. The outcomes mirrored those of Cao et al. (2022), who found that because plant extracts effectively inhibit fat oxidation and spoiling, meat treated with them had low values for the peroxide number value during cold storage. According to Fourati et al. (2020), meat's peroxide number value shouldn't be more than 25 mEqO_2 kg^{-1}

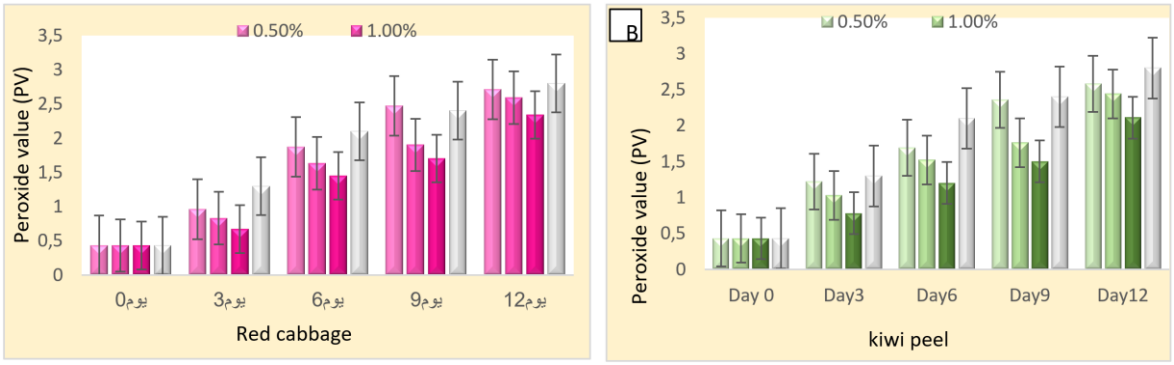

Figure 8. Peroxide Number for Minced meat patties Treated with Plant Extracts A: extract of red cabbage leaves B: kiwi peels (mean ± *SD; n* = 3). Bars with different letters differ significantly (p <0 .05).

Free fatty acids (FFAs)

Based on the findings in Figure 9 this study examines the impact of including plant extracts of cabbage and kiwi peels on the quantity of free fatty acids (FFAs) in chilled meat patties. The findings demonstrated that at the start of storage, the value of FFAs for meat patties treated with red cabbage extract was 0.25%, declining with After the storage period ended, the concentration rose by 1.5% to 0.77 which was lower than the control sample's 1.27% free fatty acid (FFAs) content. When the concentration of kiwi peel extract was raised to 1.5%, the percentage of fatty acids decreased more noticeably and at the conclusion of the storage period, the FFAS percentage was 0.96%. The presence of potent natural antimicrobial compounds that inhibit the production of the lipase enzyme secreted by certain types of bacteria involved in the enzymatic spoilage of meat is the cause of the decrease in the bacterial population that occurred during the storage period which accounts for the decrease in the percentage of free fatty acids (Al-Hamdani and Al-Noor, 2024). One indicator of spoiling during storage is the hydrolysis of fats, which is simple to measure by measuring free fatty acids (Aimen and Preetham, 2024).

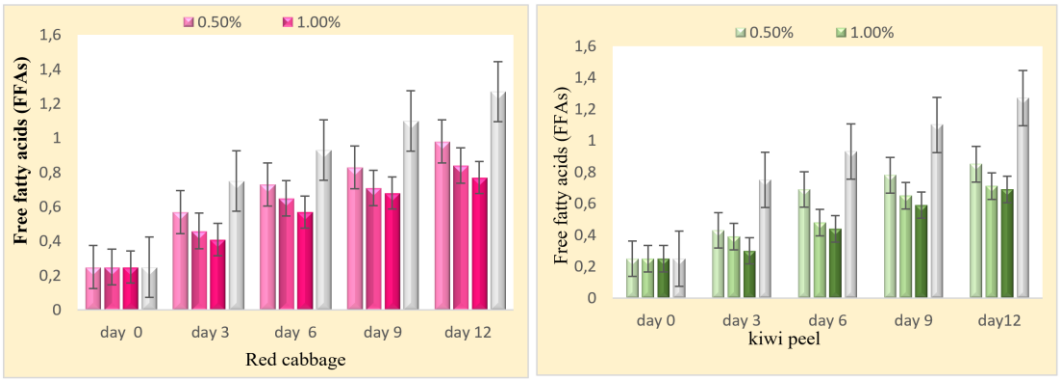

Figure 9. Determination of Free Fatty Acids (FFAs) in Minced Meat patties Treated with Plant Extracts A: Red cabbage Leaves B: Kiwi Peels (mean ± *SD; n* = 3). Bars with different letters differ significantly (p <0 .05).

Conclusion

This study demonstrated that plant extracts, particularly kiwi and beet peels, possess strong antioxidant and antibacterial properties. Beet peel extract showed the highest phenolic and flavonoid contents and the strongest free radical scavenging and iron-chelating activities. Incorporation of kiwi and red cabbage extracts into minced meat patties effectively reduced total aerobic bacteria and *Listeria monocytogenes* counts and slowed lipid oxidation during refrigerated storage. These findings indicate that selected plant extracts, especially kiwi peels, can serve as natural antioxidants and bio-preservatives in meat products, offering a safer and more sustainable alternative to synthetic additives while enhancing food safety and shelf life.

Data availability

Does not apply)

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