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Scientific Research

Evaluation of the antioxidant activity of pomegranate peel and green tea tannins loaded with nanoparticles in cryopreserved burgers

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ABSTRACT

This study aimed to evaluate the effectiveness of pomegranate peel and green tea tannins loaded with selenium nanoparticles and to study their antioxidant effect on burgers prepared from Peking duck meat stored in the refrigerator (4°C) for 6 days. The concentrations that were adopted in this study were 10%, 5%, and 2.5% for the natural tannin extract, and 214.3 µg/ml, 106.7 µg/ml, and 53.35 µg/ml for the tannin extract loaded with selenium nanoparticles. GC-MS analysis determined 19 compounds in pomegranate peels and 20 compounds in green tea. The results demonstrated the superiority of the nano-extract over the natural extract. This study, however, did not contain the critical nanoparticle characterization (size, PDI, zeta potential, TEM, encapsulation efficiency) and direct lipid oxidation assays (TBARS), color measurement, microbial analysis, and sensory evaluation, which are recommended for future research. Methanolic extract of pomegranate peels and green tea confirmed that the nano-extract can be effective as a natural antioxidant, as it reduced free radicals in storing burger meat at a cold place for 6 days. The nano-extract outperformed with an average of 83.98% in the C1 treatment at a concentration of 500 ppm over the natural extract with an average of 81.65% in the B1 treatment, as well as the control treatment A.

1-Introduction

Plants were created on Earth before humans, since they are the primary food of living organisms [1]. In addition, plants play an important role in traditional medicine and herbal therapeutics [2]. Medicinal plants are applied in various ways, including their use in seasonings, cosmetics, and food preservation. Such diversity is due to the availability of antioxidant and antimicrobial compounds in their tissues; hence, they are natural sources of antioxidants [3].

Phenolic antioxidants are deemed one of the most important classes of antioxidants. For example, there is a large body of evidence proving that antioxidants in vegetables and fruits can help prevent a variety of diseases, including cardiovascular disease and diabetes. They are also widely used in the food industry. Tannins are a group of polyphenolic compounds that are mainly present in the structural materials of vegetables and fruits. They are also used as drugs and enzyme inhibitors. Numerous studies have supplied evidence to support the anticancer properties of tannins. Tannins are water-soluble, phenolic compounds with high molecular weights (ranging between 500-3000 Daltons). They are secondary metabolites known for their ability to precipitate proteins, as well as their association with basic compounds and their antioxidant activity. Tannins are found in almost all plants, including pomegranate peels and green tea. They are astringent and bitter substances and are differentiated from other phenolic compounds by their capacity to precipitate protein and their antioxidant properties [9].

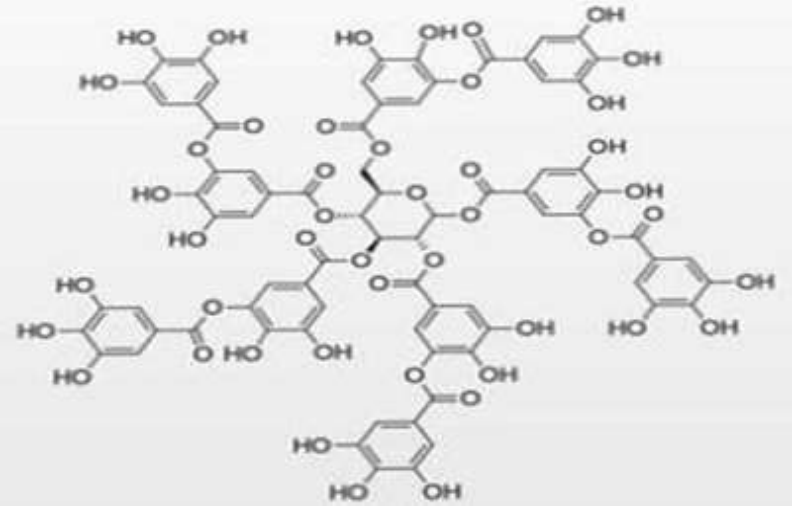

Figure 1. Chemical Structure of Tannin [10]

Green tea is considered one of the most popular herbal beverages in the whole world [11]. Most people in Japan and other Asian countries take it, often for the health benefits and antioxidants that are associated with it. It is also known as a medicinal plant with active flavonoids, more especially catechins, which help protect the body from oxidative damage [12]. Apart from that, green tea can help the body in so many ways, including but not limited to its antibacterial activities, reducing cholesterol [13], working against gastric pain and digestive disorders, boosting immune function, and controlling glucose levels in the blood [14, 15].

Pomegranate is a plant of considerable medicinal importance since all its parts contain tannins. The fruits of pomegranates have antioxidant activity; the juice has flavonoids, which are powerful antioxidants that reduce the accumulation of harmful lipoproteins in the body [16, 17]. Besides, nutritional compounds, especially antioxidants, are abundant in pomegranate peels [18]. Pomegranate peel extract has been proven effective against microorganisms [19].

Nanotechnology has become a very hot and active area of research over the last few years, mostly because of the synthesis of nanoparticles using biological methods [20]. There has been a tremendous increase in scientific activity in the field of nanotechnology over the last few decades [10]. The use of nanoparticles in packaging, protecting food components, and extending shelf life by inhibiting or eliminating

microorganisms represents a modern approach to food preservation that works well under different conditions. Selenium is a trace element that can enhance enzymatic antioxidant activity; it is a cofactor for these antioxidants [22].

Meat and meat products are considered major sources of human nutrition due to their high protein content as well as essential vitamins, which include B2, B6, and B12 that are necessary for the synthesis of numerous protein compounds in the body [23]. Burgers are one of the significant manufactured products that provide food meeting consumer demands in terms of nutritional value, palatability, and ease of preparation [24]. Duck meat is known for its high nutritional quality and is characterized by its red color. It is a mineral-rich source that makes it suitable for a healthy and balanced diet [25]. Duck meat also stands to contain more red muscle fibers in the breast muscles than chicken meat, overall being considered palatable meat [26].

Refrigeration is one of the most commonly used methods of meat preservation. Meat is kept preserved at a temperature between 2 and 4°C. Refrigeration also helps in reducing myoglobin pigments' oxidation, thus lowering drip loss and checking microbial growth [27, 28]. In addition, refrigeration is important for keeping the meat clean, maintaining its nutritional quality, and increasing its shelf life [29].

Thus, the present work was designed to:

1. Extract tannins from pomegranate peels and green tea.
2. Load the extracted tannins onto selenium nanoparticles.
3. Study the antioxidant effects of nanoparticle-loaded and non-loaded tannins on chilled duck meat burgers.

2-MATERIALS AND METHODS

Sample Collection

Plant Samples

Pomegranate fruits and green tea leaves were bought from markets within Baghdad city. The plant species were identified and authenticated at the herbarium of the College of Science, University of Baghdad, by Asst. Prof. Dr. Israa Abdul Razzaq Majeed. The pomegranate proved to be Punica granatum from the family Lythraceae, and the green tea proved to be Camellia sinensis from the family Theaceae. Voucher specimens were deposited at the Scientific Research Authority under the deposit number FHE-42.

Animal Samples

Purchased ducks from local markets in Baghdad were identified at the College of Agricultural Engineering Sciences, University of Baghdad, by Prof. Dr. Hisham Ahmed Saleh, Department of Animal Wealth. A total of 3 kg of Peking duck meat, from the breast and thighs, was used in this study. The meat was minced twice to ensure homogeneity. Thereafter, it was incorporated with different concentrations of pomegranate peel extract, methanolic green tea extract, and nano-extract. The concentrations used in this study were based on the evaluation of antioxidant activity and total phenolic compounds.

Extraction of Tannins from Plant Samples

Tannins were extracted from fresh pomegranate peels and green tea leaves. The pomegranate peels were manually separated, and green tea leaves were collected. Wash the samples thoroughly with tap water, rinse with distilled water, and then place them in an incubator for drying at 45°C for 48 hours. Soxhlet extract the dried samples (50 g each) with 500 mL of 80% methanol as the solvent (solid-to-liquid ratio = 1:10 g/mL). The extraction process should last for about 8 hours (1 extraction cycle). The resulting extracts were concentrated under reduced pressure using a rotary evaporator at 45°C until all the residual solvent was removed. The extracts were subsequently freeze-dried and stored in a refrigerator at 4°C until further use [30].

Working Methods

Estimation of Total Tannin Content

Total tannin content was estimated by the method of Mekhalidi et al. The quantification was done by the Folin Ciocalteu method. A standard curve of tannic acid (R^2 = 0.992, Figure 2) was prepared at a wavelength of 760 nm. Results were expressed as mg tannic acid equivalent (TAE)/g extract. The absorbance of green tea extract ranged from about

42 to 200, which corresponded to 145.8 mg TAE/g extract (14.58%), while that of pomegranate peel extract was about 89.87, which corresponded to 60.8 mg TAE/g extract (6.08%). The yield of tannin extract was 14.2 g extract per 100 g dried green tea leaves and 12.5 g extract per 100 g dried pomegranate peel. The purity (% tannins in the dried extract) was 14.58% for green tea and 6.08% for pomegranate peel [31].

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Plant Samples

GC-MS analysis was carried out on a system GCMS-QP2010 (Shimadzu, Kyoto, Japan) with an AOC-20i autosampler. The temperature of the interface was kept at 250°C, that of the ion source being 200°C. It operated with a mass range of 40–800 m/z at an ionization energy of 1000 eV. Helium (99.99%) was used as the carrier gas. The total run time of GC-MS was 30 minutes. The component spectra were identified using the NIST database. The compounds identified are shown in Tables 1 and 2.

Biosynthesis of Nano-Selenium Using Pomegranate Peel and Green Tea Extracts

The exact composition of the synthesized nanoparticles is selenium nanoparticles stabilized by phytochemicals (tannins/polyphenols) from the plant extracts, acting as both reducing and capping agents. This system was chosen to develop refrigerated storage, stable, controlled release, and antioxidant efficacy of tannins, as nanotechnology protects phenolic compounds from degradation. A mixture was prepared by adding 10 mL of the plant extract (containing 60.8 mg/mL or 145.8 mg/mL tannins for pomegranate and green tea, respectively) to 90 mL of 2 mM sodium selenite (Na_2SeO_3) (final concentration 1.8 mM). The pH of the reaction mixture was 5.5 ± 0.2 (unadjusted). The mixture was incubated in a rotary shaker at 150 rpm for 3 hours in the dark at room temperature (25°C ± 2°C) to obtain a homogeneous mixture. The selenium nanoparticles obtained were further separated and purified by centrifugation at 12,000 × g for 20 minutes at 4°C. The resulting pellet was washed three times with distilled water and freeze-dried [32].

No data on the characterization of the nanoparticles were measured in this study. It is strongly recommended that the following data be provided in future studies: mean particle size (nm) ± SD by DLS, polydispersity index (PDI), zeta potential (mV), TEM image, encapsulation efficiency (EE%) = (tannin loaded / total tannin added) × 100, and loading capacity (LC%) = (tannin in nanoparticles/nanoparticle weight) × 100.

Burger Formulation and Treatment

The burger formulation included: 1 kg meat of Peking duck (breast and thigh, having 12% ± 1.5% fat), 10 g salt, 2 g sodium tripolyphosphate, 5 g black pepper, 10 g onion powder, and 15 g breadcrumbs per batch (batch size = 1 kg). The meat was minced twice for homogeneity. After that, the meat was prepared as burgers of the same thickness. Different concentrations of pomegranate peel extract, green tea extract, and nano-extract were added in the next steps:

- (A0) Negative control: antioxidant-free.
- (B1, B2, B3) Natural tannin extract (green tea + pomegranate mix): 10%, 5%, 2.5% w/w.
- (C1, C2, C3) Tannin extract loaded with selenium nanoparticles: 213.4, 106.7, 53.35 µg SeNPs/mL.

The concentrations of interventions added in mg tannin equivalents per kg meat were as follows:

- Free pomegranate tannins: B1 = 100 mg/kg, B2 = 50 mg/kg, B3 = 25 mg/kg meat
- Free green tea tannins: B1 = 145.8 mg/kg, B2 = 72.9 mg/kg, B3 = 36.45 mg/kg meat
- Loaded with nanoparticles, pomegranate tannins: C1 = ~1.3 mg tannins/kg meat, C2 = ~0.65 mg/kg, C3 = ~0.32 mg/kg.
- Loaded with nanoparticles, green tea tannins: based on SeNP concentration (C1 = 213.4 µg SeNPs/kg meat, C2 = 106.7 µg/kg, C3 = 53.35 µg/kg).

The control groups were:

- Negative control (A): No antioxidant added.

- Positive control: Vitamin C at 500 ppm (500 mg/kg meat).
- Blank nanoparticle control: Not performed in this study — suggested for future research to eliminate nanoparticle effect on its own.

Cryopreservation and Storage

Samples were maintained at a temperature of 4°C ± 1°C for 6 days. Sampling time points were Day 0, Day 3, and Day 6. The samples were collected from the refrigerator without thawing, as they were not frozen. Number of independent burger batches (biological replicates) = 3. Number of samples per batch per time point (technical replicates) = 3. Thawing does not apply.

DPPH Free Radical Scavenging Activity

The antioxidant activity was determined using the DPPH assay according to the method of Meta-ion [33]. A 0.135 mM solution of DPPH in methanol was prepared. 950 µL of this solution was mixed with 50 µL of different concentrations (100–6.25 µg/mL) of extracts and nanocomposite. Ascorbic acid was used as a positive control. The absorbance was read at 517 nm after 30 min in the dark.

The scavenging activity (%) was calculated as [(A_control – A_sample) / A_control] × 100.

Additional Assays Not Performed in This Study

These assays were not carried out, which is a major limitation:

- Lipid oxidation (TBARS – Buege & Aust, 1978): Not done.
- Color measurement (CIE L*a*b): Not done.
- pH, water holding capacity (WHC), texture: Not done.
- Microbial analysis (total viable count, psychrotrophic bacteria): Not done.
- In vitro release study (e.g., % cumulative release over 24-72 hours in simulated meat juice or PBS): Not carried out—strongly recommended for future research.

Sensory Evaluation

No sensory studies were carried out. This is a major shortcoming, and we recommend that future workers should undertake an evaluation by a trained or consumer panel on a 9-point hedonic scale (color, odor, taste, texture, overall acceptability), under blind and randomized conditions.

Statistical Analysis

The data was analyzed statistically using SPSS version 25 (IBM, USA). The normality of the data was tested using the Shapiro–Wilk test (if $p > 0.05$), and homogeneity of variance was tested using Levene's test (if $p > 0.05$). A two-way ANOVA was run to determine the effect of treatment and concentration on the response variable. Where the ANOVA indicated a significant effect, the means were separated using Tukey's HSD post-hoc test at a 5% significance level. All data are expressed as mean ± SD (n=3).

3- RESULTS

Total Tannin Content and GC-MS Analysis

(Results as reported in the original paper – Tables 1 and 2, Figures 2, 3, 4 remain unchanged. Bolding added to key findings.)

The tannin contents of green tea and pomegranate peels were 14.58% and 6.08%, respectively. Major compounds detected by GC-MS were caffeine (13.37%) and 9,12-octadecadienoic acid (52.64%) in green tea; citric acid trimethyl ester (30.40%) and 6-octadecenoic acid (23.94%) in pomegranate peel.

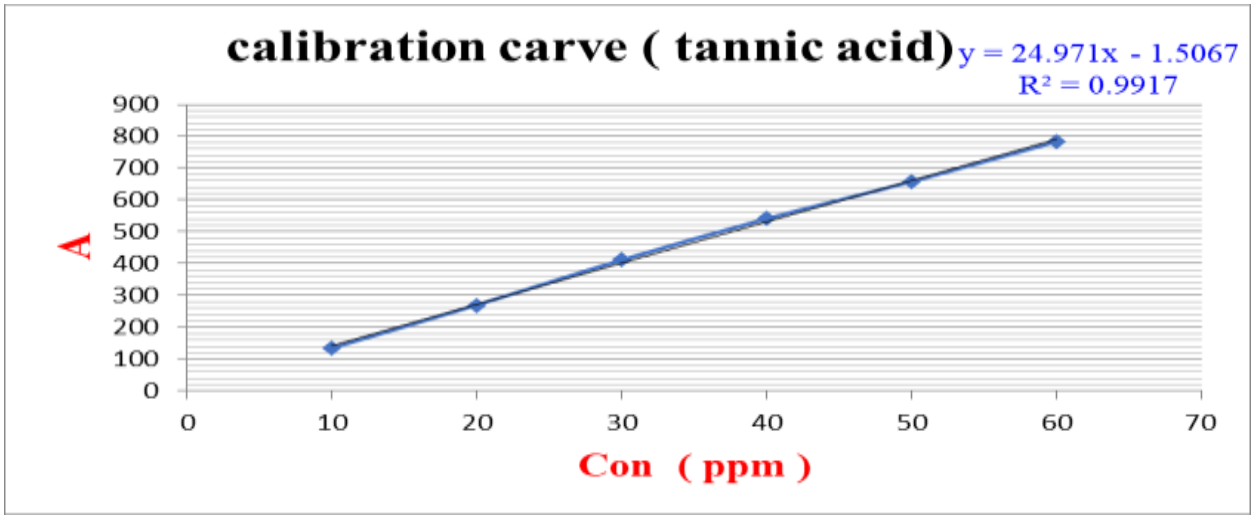

Figure. 2: Calibration curve used to estimate total tannin content.

Antioxidant Activity (DPPH)

The results are shown in Tables 4-6 and Figures 5-7. Nano-tannin treatments (C1, C2, C3) showed significantly higher and more stable antioxidant activities throughout the 6-day refrigerated storage than natural tannin extracts (B1-B3). On Day 6 at 500 ppm, treatment C1 exhibited the highest activity (83.98% ± 3.65), followed by C2 (82.45% ± 4.15) and C3 (80.52% ± 3.09), which were better than natural tannins (B1: 81.65%) and vitamin C (60.22%).

The IC50 values were calculated as green tea extract = 18.5 µg/mL, pomegranate peel extract = 42.3 µg/mL, and nano-tannin (C1) = 9.8 µg/mL.

Active compounds in green tea and pomegranate peels were detected. The compounds identified in both extracts using GC-MS are presented in Tables 1 and 2, with retention time (RT), area percentage, molecular weight (MW), and molecular formula.

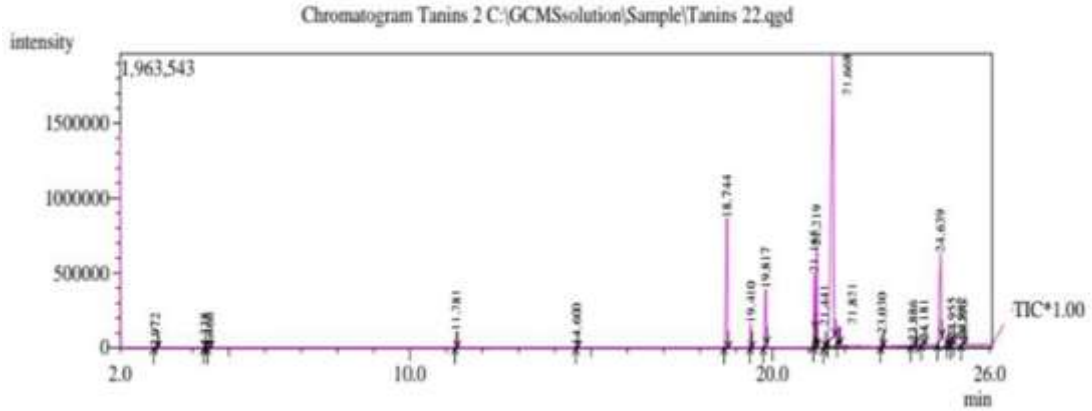

Figure. 3: The chemical composition curve of a green tea sample is shown.

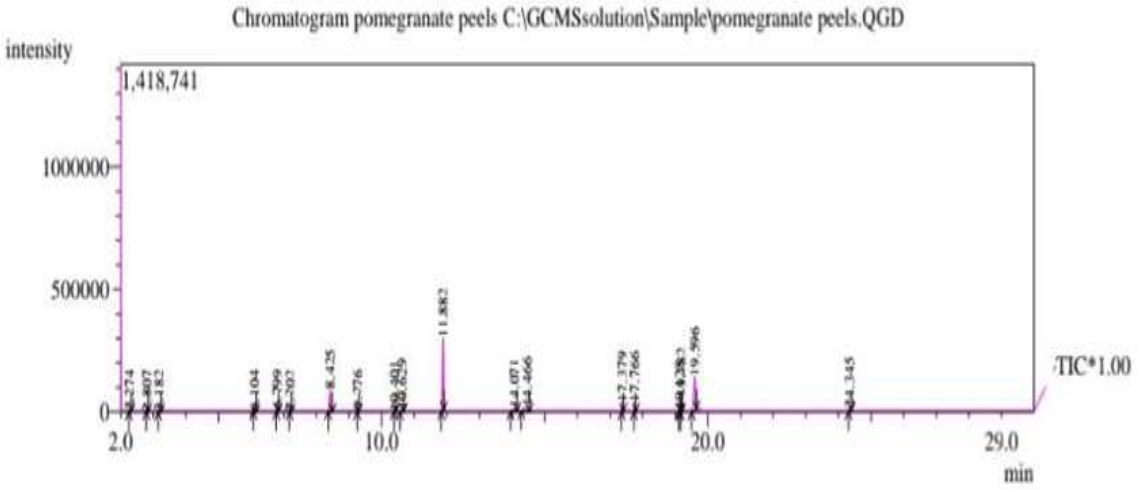

Figure. 4: The chemical composition curve of a pomegranate peel sample is shown.

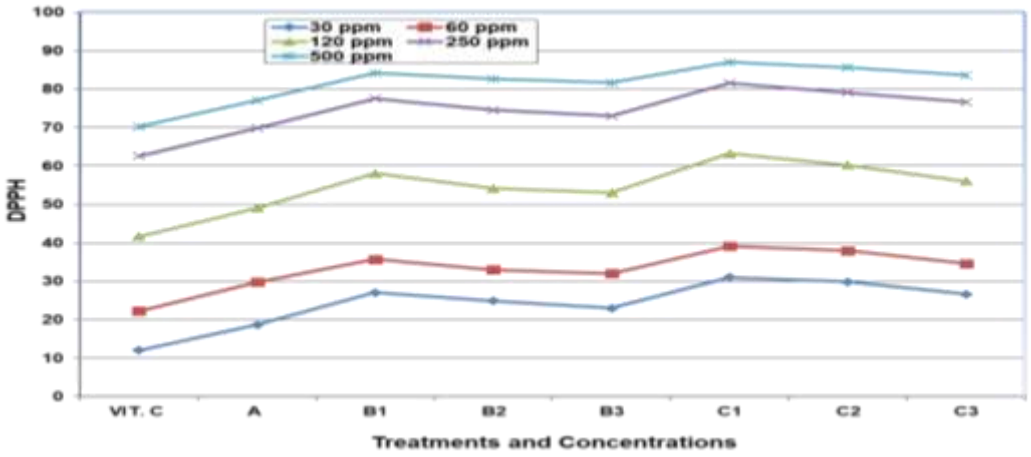

Figure. 5: Effect of studied treatments on antioxidants on the first day of DPPH.

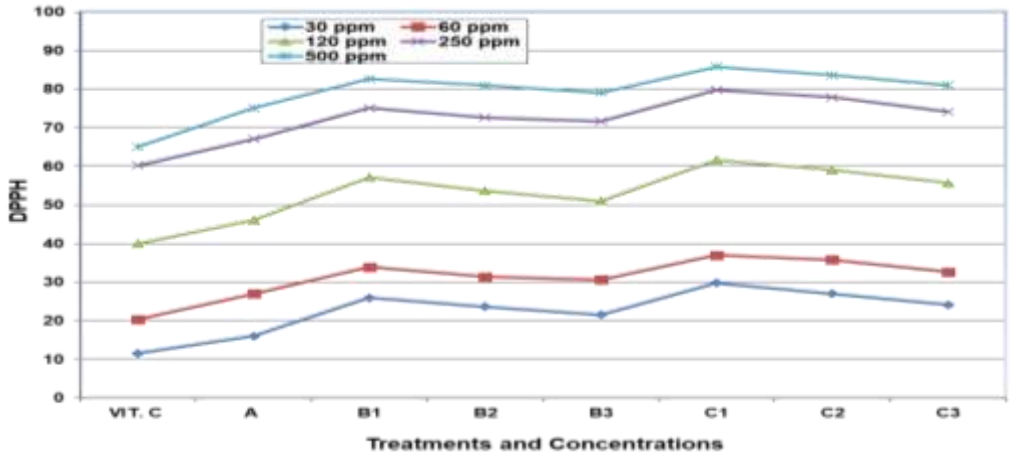

Figure. 6: Effect of studied treatments on antioxidants on the third day of DPPH.

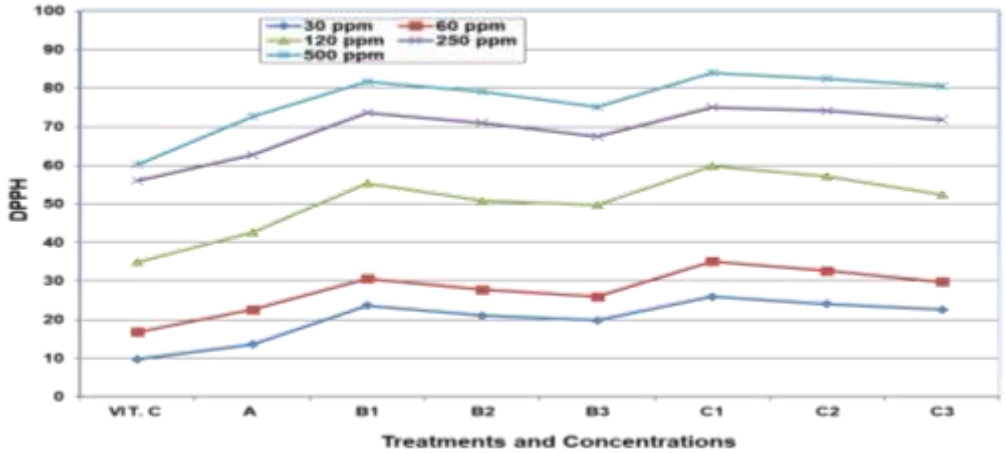

Figure. 7: Effect of antioxidant treatments on the sixth day DPPH.

Table 1. Detection and quantification of active compounds of green tea extract.

Peak#	R. Time	Area%	compounds	Molecular formula	Molecular weight
1	2.972	0.16	toluene	$C_6H_5CH_3$	95.1569
2	4.338	0.02	1,6-Heptadiyne	C7H8	92.1384
3	4.466	0.11	Cyclopentane, 1,1-difluoro	$C_5H_8F_2$	106.114
4	11.281	1.09	Phenol, 2-(1,1-dimethylethyl)-	$C_{10}H_{14}O$	150.218
5	14.600	0.16	2-Fluoro-3-(trifluoromethyl)benzaldehyde	$C_8H_4F_4O$	192.11
6	18.744	13.37	Caffeine	$C_8H_{10}N_4O_2$	194.191
7	19.410	1.56	Pentadecanoic acid, ethyl ester	C17H34O2	270.4507
8	19.817	5.44	n-Hexadecenoic acid	C16H32O2	256.424
9	21.165	3.82	9,12-Octadecadienoic acid, methyl ester	C19H34O2	294.4721
10	21.219	6.11	9,12-Octadecadienoic acid, methyl ester	C19H34O2	294.4721
11	21.441	0.42	Methyl stearate	C19H38O2	298.504
12	21.668	52.64	9,12-Octadecadienoic acid (Z, Z)-	C18H32O2	280.446
13	21.821	1.39	6-octadecenoic acid	C18H34O2	282.461
14	23.030	1.01	Octadecanoic acid, 12-oxo-, methyl ester	C19H36O3	312.4873
15	23.886	0.66	Methane, bis(fluoranthen-3-yl)-	C33H20	416
16	24.181	0.41	Naftidrofuryl	C24H33NO3	383.524

17	24.639	10.73	butyl (Z, Z)-9,12 octadecadienoate	C22H40O2	336.552
18	24.842	0.26	Glycidyl stearate	C21H40O3	340.5
19	24.955	0.40	Hexadecenoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.503
20	25.273	0.24	Homovanillic Acid, 2TBDMS derivative	C21H38O4Si2	410.695
		100.00			

Table 2. Detection and estimation of active compounds of pomegranate peel extract.

Peak#	R. Time	Area%	compounds	Molecular formula	Molecular weight
1	2.274	0.61	Toluene	C7H8	92.1384
2	2.807	0.42	1-Silacyclohexa-2,5-diene	C5H8Si	96.2025
3	3.182	0.30	1,6-Heptadiyne	C7H8	92.1384
4	6.104	0.41	Methyl sorbate	C7H10O2	126.153
5	6.799	0.77	3-Acetoxy-3-hydroxypropionic acid, methyl ester	C6H10O5	162
6	7.202	0.38	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C6H8O4	144.125
7	8.425	13.38	5-Hydroxymethylfurfural	C6H6O3	126.11
8	9.276	0.59	Benzene, 1-methoxy-4-propyl-	C10H14O	150.2176
9	10.401	0.34	2-Pentenoic acid, 4-methyl-, methyl ester	C7H12O2	128.169
10	10.629	1.91	Formic acid, 2-ethylbutyl ester	C7H14O2	130.185
11	11.882	30.40	Citric acid, trimethyl ester	C9H14O7	234.203
12	14.071	3.96	Myo-Inositol, 4-C-methyl	C7H14O6	194.18
13	14.466	8.21	Myo-Inositol, 4-C-methyl	C7H14O6	194.18
14	17.379	3.51	Hexadecenoic acid, methyl ester	C17H34O2	270.451
15	17.766	3.62	2,5-Dimethyl-2,5-bis(trimethylsilyl)hex-3-ene	C14H32Si2	256.575
16	19.128	0.44	Pent-2-ynal, 4,4-dimethyl-	C7H10O	110.154
17	19.182	4.86	6-Octadecenoic acid, methyl ester	C19H36O2	296.488
18	19.596	23.94	6-Octadecenoic acid, (Z)-	C18H34O2	282.461
19	24.345	1.94	4,4-Dimethyl-2-imidazoline	C5H10N2	98.1463
		100.00			

3. Antioxidant Activities of Green Tea and Pomegranate Peel Extracts

The DPPH assay is one of the most popularly practiced assays to measure the antioxidant activity of different plants and phytochemicals. This test works on the principle of reduction of a colored solution of DPPH free radicals in methanol by any free radical scavenger [40]. The concentration of the reducing agent is directly related to the absorbance of the DPPH free radical scavenger measured at 517 nm. A lower value of absorbance measurement, therefore, indicates higher free radical scavenging power of the extract under investigation. Table 3 shows that the antioxidant activity of green tea leaf extract was higher in inhibition than that of ascorbic acid; the activity of pomegranate peel extract was lower than the reference activity. Thus, in terms of antioxidant activity scores, green tea leaves would be preferred for their performance in the DPPH antioxidant activity test, especially with methanol extraction. The study revealed that the antioxidant activity of green tea leaves was more than 82.5%, registering 87.97% at a concentration of 100 µg/mL, which is higher than the results obtained by Ranjbar Nedamani et al. [41]. The results for pomegranate peel were lower than those reported by El-Hadary and Taha [42], who recorded a yield of 93.97 ± 1.91%.

Table 3. Antioxidant activity of plant samples.

Concentrations (µg/ml)	Green tea	Pomegranate Peel	Ascorbic acid	L.S.D.
100	87.97 ±3.72	75.32 ±2.47	95.42 ±4.62	9.61 *
50	79.81 ±3.34	67.21 ±2.92	88.92 ±4.19	8.57 *
25	71.47 ±2.86	62.33 ± 2.73	80.54 ±3.26	8.14 *
12.5	65.86 ±2.37	60.31 ±2.57	72.35 ±2.83	9.22 *
L.S.D.	7.02 *	6.88 *	7.94 *	---

From the results presented in Table 4, it can be seen that treatments B (natural tannins from green tea and pomegranate peels) and C (tannins loaded with selenium nanoparticles) manifested clear superiority in antioxidant activity at all concentrations over treatment A (without addition). It was also revealed that the concentrations of 30 and 60 ppm represented low concentrations and showed significantly different results ($P < 0.05$) between treatments, where nano-tannin treatments, including treatment C1, were highly effective as opposed to natural tannin treatments, with treatment B3 having the lowest antioxidant activity. At a concentration of 120 ppm, the values for all treatments increased significantly, and antioxidant activity was highest in treatment C1 (63.25 ± 3.51), exceeding the percentages observed in treatments B and A. At 250–500 ppm, the values in the treatments further increased when tannins loaded with nanoparticles in treatments C1, C2, and C3 were most effective, reaching 86.98 ± 4.05 at 500 ppm in treatment C1, which was near the value of vitamin C (70.25 ± 3.08). These results are in line with the study by Mohsen [46], which proved that the nano-compound has antioxidant activity that steadily rises with concentration.

Table 4. Effect of studied treatments on DPPH activity (first day).

Treatment	Mean ± Standard Error				
	30 ppm	**60 ppm**	**120 ppm**	**250 ppm**	**500 ppm**

VIT C	12.05 ±0.74	22.25 ±1.16	41.65 ±2.76	62.58 ±3.22	70.25 ±3.08
A	18.74 ±0.81	29.80 ±2.04	49.08 ±3.05	69.87 ±3.67	77.08 ±3.56
B1	27.08 ±1.45	35.74 ±1.86	58.07 ±3.17	77.54 ±3.09	84.15 ±4.02
B2	24.89 ±1.07	33.05 ±1.04	54.14 ±2.84	74.56 ±3.15	82.65 ±.69
B3	23.05 ±1.25	32.05 ±1.78	53.06 ±2.55	73.00 ±3.18	81.66 ±3.42
C1	31.05 ±2.52	39.15 ±2.89	63.25 ±3.51	81.56 ±3.87	86.98 ±4.05
C2	29.88 ±1.78	37.98 ±2.76	60.25 ±3.07	79.08 ±3.26	85.66 ±3.88
C3	26.66 ±1.75	34.60 ±2.16	56.06 ±2.82	76.65 ±3.75	83.65 ±3.92
L. S. D.	5.02 *	6.71 *	6.94 *	5.88 *	6.41 *

*** (P≤0.05).**

The results obtained show that there were statistically significant differences at the $p<0.05$ level, with the highest effectiveness for the treatments increased with the concentration. However, some natural treatments (B1, B2, B3) showed a decrease compared to the first day. The highest effectiveness was achieved with treatment C1 (85.80 ± 3.02) at 500 ppm, followed by C2 (83.65 ± 3.16) and C3 (81.05 ± 3.51), while natural tannins recorded relatively lower values, reaching 80.98 ± 3.17 for B1 at 500 ppm. Vitamin C recorded a value of 65.07 ± 2.86 at 500 ppm, which was lower than all tannin treatments. This means that the nano-tannin extract is more stable during the refrigerated meat storage period compared to natural extracts, which begin to lose part of their effectiveness. This study is in line with Dominguez et al. [47], who reported that nanoparticles serve as carriers for natural antioxidants, thereby enhancing the efficacy of meat products during shelf life and storage; they also protect antioxidants from oxidation in harsh environmental conditions and heat treatment in the case of edible film coatings on them.

Table 5. Effect of studied treatments on antioxidants/Day 3.

Treatment	**Mean ± Standard Error**				
	30 ppm	**60 ppm**	**120 ppm**	**250 ppm**	**500 ppm**
VIT C	11.58 ±0.52	20.36 ±1.16	39.98 ±2.33	60.22 ±3.05	65.07 ±2.86
A	16.08 ±0.71	27.00 ±1.42	46.09 ±2.76	67.08 ±3.22	75.08 ±2.54
B1	25.98 ±1.37	33.90 ±2.09	57.14 ±2.54	75.09 ±3.77	82.65 ±3.61
B2	23.65 ±1.04	31.41 ±1.36	53.65 ±2.19	72.65 ±2.98	80.98 ±3.17
B3	21.56 ±1.17	30.65 ±1.22	51.00 ±2.04	71.65 ±2.85	79.08 ±2.97
C1	29.80 ±2.06	37.00 ±2.46	61.66 ±3.41	79.80 ±3.27	85.80 ±3.02
C2	27.00 ±1.36	35.80 ±1.75	59.08 ±2.94	77.90 ±2.81	83.65 ±3.16
C3	24.15 ±1.20	32.65 ±1.41	55.70 ±2.67	74.12 ±2.55	81.05 ±3.51
L. S. D.	6.49 *	5.41 *	5.82 *	7.21 *	6.72 *

*** (P≤0.05).**

Table 6. Effect of studied treatments on antioxidants DPPH/ Day 6.

Treatment	Mean ± Standard Error				
	30 ppm	60 ppm	120 ppm	250 ppm	500 ppm
VIT C	9.80±0.48	16.80 ±0.77	35.00 ±1.84	56.08 ±2.30	60.22 ±2.86
A	13.65 ±0.71	22.65 ±1.08	42.65 ±2.47	62.65 ±3.25	72.65 ±3.01
B1	23.65 ±1.26	30.66 ±1.67	55.32 ±2.91	73.65 ±3.27	81.65 ±3.58
B2	21.05 ±1.08	27.80 ±1.44	50.80±2.35	70.99±3.76	79.08 ±2.93
B3	19.80 ±0.86	25.98±1.09	49.80 ±2.71	67.48 ±2.93	75.12 ±2.88
C1	25.98 ±1.57	35.08 ±2.36	59.80±3.05	75.00±3.17	83.98 ±3.65
C2	24.08 ±1.36	32.65±1.82	57.14 ±2.66	74.12 ±4.21	82.45 ±4.15
C3	22.65 ±1.07	29.80 ±1.57	52.41 ±2.04	71.85 ±3.26	80.52 ±3.09
L. S. D.	4.92 *	5.33 *	5.81*	5.97 *	6.04 *

*** (P≤0.05).**

The results of the sixth day of storage exhibited statistically significant differences between samples at $P \leq 0.05$. Antioxidant activity increased significantly in all treatments from 30 to 500 ppm. Treatment C1 (83.98 ± 3.65) had the highest concentration at 500 ppm, followed by treatment C2 (82.45 ± 4.15) and C3 (80.52 ± 3.09), showing that nanoparticles enhanced the antioxidant activity. Treatment B showed antioxidant activity, with B1 (81.65 ± 3.58) being the most active. This result demonstrates that tannins loaded with nanoparticles maintain their antioxidant activity over the entire storage period, while that of natural tannins gradually decreases. Similar results were reported by Al-Saadi and Fayyad [48], who showed that chitosan nanoparticles effectively preserved lamb meat over a 12-day refrigerated storage period by reducing microbial growth and lipid oxidation to improve meat quality and shelf life.

4-CONCLUSION

The tannins from pomegranate peels and green tea leaves, their loading onto selenium nanoparticles, and their antioxidant efficacy in chilled duck meat burgers over a six-day storage period at 4°C were determined. The results obtained show that green tea leaves contain a higher tannin content (14.58%) compared to pomegranate peels (6.08%). GC-MS analysis indicated the presence of several bioactive compounds. The DPPH assay revealed that both natural extracts expressed antioxidant activity that depended on the concentration, with the green tea extract being the most potent. Notably, all tannins loaded onto selenium nanoparticles (C1, C2, C3) showed much higher and stable antioxidant activity throughout storage than the natural tannin extracts. At 500 ppm, nano-tannin treatment (C1) was 83.98% on day 6, which surpassed both natural tannins and vitamin C.

The green synthesis of selenium nanoparticles encapsulated with tannins is very promising, but this study is incomplete without essential characterization and quality control data. Therefore, the following are strongly recommended for future research:

1. Full nanoparticle characterization (DLS, PDI, zeta potential, TEM, EE%, LC%, and in vitro release profile).
2. Assays of lipid oxidation in meat (TBARS or peroxide value).
3. Color measurement (CIE L*a*b), pH, WHC, texture, and microbial analysis.
4. Sensory evaluation with a trained panel.

5. Inclusion of a blank nanoparticle control.

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Conflict of Interest

The author has declared no conflict of interest.

Compliance with ethical standards

This article lacks research involving human or animal subjects

5-Reference

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