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Fourier Transform Infrared Spectroscopy and Gravimetric Analysis of Protein Denaturation in Egg Albumen Treated with *Colocasia esculenta* L. Schott (Taro) Ethanolic Leaf Extract

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Abstract. One of the leading factors contributing to the development of neurodegenerative disorders like Alzheimer's and Parkinson's disease is protein misfolding. When this occurs, these proteins lose their proper form, thereby leading to dysfunction and aggregation. Limited remedies that focus on symptomatic relief have been developed, thereby leading to plant-derived compounds gaining attention as their potential to stabilize proteins. The study aims to determine taro leaf extract's effect combined with egg albumen on protein structures observed through infrared spectroscopy, evaluating protein structural change and mass measuring for protein denaturation. Researchers compared four concentrations (12.5, 25, 50, and 100%) to diclofenac sodium and distilled water, with all groups containing egg albumen. Only 100% concentration and control groups were analyzed using FT-IR spectra, revealing hydrogen bonding interactions and structural differences by distinct shifts in Amide I bands. Through deconvolution, 100% concentration showed the highest α-helix content, presence of β-sheet, and no random coil signals, revealing preservation of native secondary structure and protein stability in opposite to negative control. The gravimetric analysis revealed increased mass in higher extract concentrations. A one-way ANOVA for gravimetric analysis yielded a p-value of 7.11 × 10^ (-5), indicating significance between groups. Tukey's HSD post hoc test showed significance between 100% and the concentrations (12, 25, and 50%) with p-values of 0.013, 0.016, and 0.017, indicating distinct aggregation caused by 100% compared to the lower concentrations. Moreover, no significant differences were compared to controls with p-values of 0.149 and 0.584, suggesting aggregation and coagulative activity. Findings suggest that the extract exhibited secondary protein structural stabilizing effects on α-helix and β-sheet structures, potentially preventing misfolding despite presence of aggregation. However, further investigation is needed to evaluate its role as a coagulative agent and to understand its dosage-dependent behavior, offering valuable insights for future therapeutic approaches to protein misfolding-related conditions.

Keywords: Alternative plant-based therapeutics; Fourier transform infrared spectroscopy; Gravimetric analysis; Protein denaturation; Protein-protein interactions; Taro leaf extract.

1.0 Introduction

Protein misfolding is one of the leading factors contributing to the development of $A\beta$ amyloidosis and neurodegenerative disorders, leading to 121,499 deaths globally in 2023 from Alzheimer's disease alone. This makes it the 6th leading cause of death worldwide (Amer-Sarsour & Ashkenazi, 2019; Gadhave et al., 2024). There are three main reasons why proteins misfold. First, genetic mutations can change a protein's shape, making it unstable or sticky. A mutation in the huntingtin gene causes it to form long, tangled chains that clump together in Huntington's disease (Ross & Tabrizi, 2010). Second, the body's system for checking and mending proteins weakens as people age. Helper proteins, called molecular chaperones, become less effective as the proteasome slows down like a cell recycling system, causing misfolded proteins to build up. Third, environmental factors, such as toxins, infections, or oxidative stress, can cause proteins to fold incorrectly such as exposure to pesticides, which has been linked to the clumping of alpha-synuclein in Parkinson's disease (Tanner et al., 2011). Overall, these proteins lose their proper form, thereby leading to dysfunction, aggregation, and the formation of harmful amyloid clot deposits in the tissues and organs, further contributing to these diseases.

To address this, researchers were exploring other approaches to address protein misfolding. One method involves stabilizer drugs, such as tafamidis—which help proteins stay in their correct shape and prevent misfolding—, and antibody therapy like lecanemab, for Alzheimer's disease (Maurer et al., 2018). These lab-made antibodies attach to misfolded proteins, such as amyloid-beta, marking them for removal by the immune system (Van Dyck et al., 2022). Researchers are also working on drugs that improve the cell's natural cleaning process. For example, rapamycin activates autophagy, a process where cells break down and remove harmful protein clumps (Rubinsztein et al., 2012). Moreover, gene therapies, including CRISPR gene editing, offer hope by fixing mutations that cause misfolding, which may help treat conditions like Huntington's disease (Alkanli et al., 2022). However, many drugs have difficulty reaching the brain, and some only show a slowdown in symptoms rather than curing the disease. As an alternative, in vivo studies reveal that natural compounds, specifically phenolic and non-phenolic compounds, may be a promising approach as potential therapeutics, as naturally occurring substances have been shown to inhibit protein misfolding directly.

In a study conducted by (Cho & Kang, 2017), an in-vitro Parkinson's disease model, taro extract promoted neurite outgrowth, enhanced synaptic connections, and upregulated genes linked to neuroplasticity (e.g., GAP-43), suggesting axonal regeneration and neuronal survival as taro leaf extract can demonstrate neuroprotective potential relevant to neurodegenerative diseases. It also reduced neuroinflammatory markers like iNOS and IL-6, which are implicated in dopamine neuron degeneration. The extract's flavonoids and triterpenoids contribute to antioxidant and anti-inflammatory effects, combating oxidative stress, a key factor in neurodegeneration (Lad et al., 2023). Additionally, taro mucilage's polysaccharides exhibit anti-inflammatory activity, potentially mitigating chronic inflammation in conditions like Alzheimer's. The mentioned traditional uses as a nervine tonic further align with its neuroprotective properties (Tosif et al., 2022). However, few studies have pushed its final drug development phase, using only its isolated bioactive compounds due to its high oxalate content, which can be considered toxic due to oxidative stress. Ongoing research further investigates these properties and their alternative applications and implications in proteins, often using in vitro models such as egg albumen.

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The egg albumen is a frequently used model for studying the antioxidant and anti-inflammatory properties of plants, as its proteins have the capability to undergo a process called protein denaturation. It is when these enzymes aggregate or clump after exposure to stress factors like heat, similar to how an egg solidifies during cooking. This mechanism allows researchers to assess the ability of substances to prevent protein denaturation by stabilizing the egg albumen's proteins, particularly ovalbumin, accounting for 54% of its protein content. (Kumarasinghe et al., 2018). Protein denaturation allows egg albumen to be an ideal model for studying Protein-Protein Interactions (PPI), paving the way for its application in neurodegenerative disease detection or observation, reducing costs, time, and reliance on live animal models for bioactive assessment (M et al., 2023). In this study, egg albumen provides a cost-effective and safer approach to investigate the effects of taro leaf extract on protein denaturation. Using Fourier Transform Infrared (FTIR) Spectroscopy, the research aims to identify changes in the functional groups and secondary protein structures (specifically the Amide I and II bands) of chicken egg albumen with taro leaf extract, diclofenac sodium, and distilled water as controls. Gravimetric analysis complements and quantifies protein precipitation as a direct measure of denaturation inhibition.

Different standard tests will be used in the study to investigate the direct protein interactions further. Fourier-transform infrared (FTIR) spectroscopy is a test that acquires emission spectra—a spectrum of frequencies of electromagnetic radiation or infrared absorption from solid, liquid, or gas samples, which analyzes protein changes in this study (Onyeaka et al., 2022). It investigates a sample's key functional groups and secondary protein structures at a molecular level. Identifying such groups is determined by the peak, number of components in a mixture, and the quality or consistency of a sample, each of which can be obtained from the raw data recorded by the machine through numbers and graphs (Jafari et al., 2017). The Amide I and Amide II bands, which correspond to C=O stretching and N-H bending vibrations, provide valuable information on protein secondary structures, such as α-helices and β-sheets, which can determine how much the structural integrity is maintained, especially after going through protein denaturations. A shift to lower wavenumbers in these bands typically indicates structural rearrangements that may promote aggregation rather than stability. The lower these numbers are, the weaker the chemical bonds that make up the protein's structure.

Although there are few studies on how taro leaf extracts affect protein interactions, their rich natural compounds make them worth studying for their role in protein stability. This study builds on past research about plant extracts and protein interactions by testing how taro leaf extracts affect egg albumen proteins. Using methods like Fourier transform infrared spectroscopy, this research will examine how taro leaf compounds change protein structures, helping to understand their effects better. Moreover, gravimetric analysis further complements these findings by quantifying protein precipitation and potential aggregation, which helps assess the effectiveness of taro leaf extract in maintaining protein stability. By exploring protein-protein interactions in taro leaf extract-treated egg albumen after denaturation, this study aims to provide new insights into using natural protein stabilizers to prevent protein misfolding where it could contribute to a deeper understanding of developing remedies for neurodegenerative diseases, preventing them from happening by targeting the root problem.

2.0 Methodology

2.1 Research Design

An area of interest was systematically investigated using a quantitative research design. The approach used numerical data and statistical analysis to test theories and produce results. It also allowed the researchers to determine connections between variables and draw conclusions about the effects of the changed variables. One-way ANOVA and Tukey's HSD post-hoc test were used to evaluate the significance of observed differences. In line with the quantitative and true experimental post-test research design, the researchers conducted four experimental groups, taro leaf extract (12.5, 25, 50, and 100%), which were compared to the positive (diclofenac sodium) and negative control (distilled water) where all groups were subjected using egg albumen model, a mixture of proteins found in egg whites for observing protein denaturation similar to cooking the egg. At the end of the study, the objective was to provide an in-depth understanding of how taro leaves affect the protein structure and interaction using the egg albumen model and its analysis through Fourier transform infrared spectroscopy and gravimetric analysis on protein denaturation.

2.2 Research Locale

The study was conducted at San Beda University-Rizal, in the Chemistry Laboratory, where the necessary apparatus like test tubes, analytical balance, and water baths are officially available. This is also where taro leaves and egg albumen from egg whites were extracted, followed by incubation to induce protein denaturation. On the other hand, Fourier transform infrared spectroscopy was performed at the Department of Science and Technology-Industrial Technology Development Institute at Taguig, where laboratory professional handling was ensured to observe protein interaction accurately.

2.3 Research Participants
No live subjects were used in the study. The researchers used a chicken egg albumen model where each sample was separated into experimental groups that were combined with different concentrations of taro leaf extract (12.5, 25, 50, and 100%). The positive control was combined with diclofenac sodium, and the negative control group received no treatment other than distilled water.

2.4 Research Instrument
This study used laboratory equipment, such as analytical balance, to determine the mass of protein denaturation of groups. A Fourier-transform infrared (FTIR) spectroscopy was used to determine the protein interactions of egg albumen with the experimental, positive, and negative control groups. Since the values perpendicular to the wavenumber regions sent by DOST came in transmittance form, absorbance values were calculated using the following equation:

$$A = -log_{10}\left[\frac{T}{100}\right]$$

A is denoted as the absorbance, and $[T/100]$ is the percent transmittance

To quantify the secondary protein structures, specifically the α-helices, β-sheets, and random coils, deconvolution was done through Origin Pro using the Gaussian function equation found below for the analyzation of peaks found in the Amide I regions of the different samples:

$$y = y_0 + A \times e^{-\frac{(x-x_c)^2}{2w^2}}$$

y represents the measured signal (such as absorbance), $y0$ is the baseline offset, A denotes the peak's amplitude which corresponds to the absorbance at its maximum, x is the independent variable (like wavelength), x_c marks the center of the peak, and w indicates its width.

Deconvolution separates overlapping peaks to help indicate the area for the respective secondary structures. After obtaining the area under the different peaks, the secondary protein structure was assigned to the pertaining region where the final area percentage was calculated using the following equation:

$$Percentage\ of\ structure\ (\%) = \left(\frac{area\ of\ individual\ peak}{total\ area\ of\ Amide\ I\ region}\right) \times 100$$

2.5 Data Gathering Procedure
Materials
The researchers collected five kilograms of taro leaves from Aurora Subdivision, Angono, Rizal. A sample is sent on-site for authentication to the Institute of Biology, University of the Philippines, Diliman. Furthermore, six eggs were bought from a local market in the same area.

Ethanol Extract of Taro Leaves
The study by Lindawati (2018) will be used as the basis for the ethanolic extraction of taro leaves. Ten kilograms of taro leaves were washed, drained, and cut into small pieces for sun drying where they were covered with a black cloth for three days. After drying, the leaves were ground into a fine powder, where 600 g were macerated with 3000 mL of ethanol with a 70% concentration, allowing enough contact time for the solvent and the bioactive compounds for proper extraction, unlike higher concentrations that evaporate too quickly. The solution is stirred every 24 hours and filtered with a cheesecloth. The filtrate was separated and mixed with a fresh solvent for re-maceration where the final filtrate was then extracted using a rotary evaporator. Afterward, it was stored inside

an amber bottle and refrigerated at 4°C.

Two-Step Fold Serial Dilution

Different batches of the extract from the ethanolic extraction were used to obtain the various concentrations (12.5, 25, 50, and 100%) of taro leaf extract through two-step fold serial dilution using the procedure of Panganiban et al. (2024) where the solution is reduced by half for each step. It allowed the researchers to determine the minimum effective concentration, maximum tolerated concentration, or the optimal dosage for a specific protein effect. No distilled water was added to the 100% concentration. For the 50% concentration, equal volumes of solute and solvent were used; for 25%, one part solute to three parts solvent; and for 12.5%, three parts solute to seven parts solvent.

Egg Albumen Extraction

The researchers separated the egg whites from the egg yolks and collected a total of 50 mL of egg whites. Using a 1:10 ratio, 1 mL of egg whites was mixed with 10 mL of distilled water. It was stirred until the solution became less viscous, and when precipitate formed, it was removed. The solution was distributed into eighteen 15 mL conical tubes, with three trials per group, each using a 0.5:4.5 additive-to-albumen ratio, and centrifuged for 24 minutes at 4000 rpm. Afterward, the precipitate (ovoglobulin) was removed through decantation, leaving the egg albumen extract in the tube (Sookhoo, 2015).

Protein Denaturation Induction

All extracted egg albumen were separated into different tubes with a 0.5 mL additive to 4.5 mL egg albumen. Each group contained three tubes of different concentrations of taro leaves and controls which were subjected to a water bath using incubation periods at 37 ± 2°C for 15-30 minutes to mirror the physiological relevance of the human body temperature. (Madhuranga & Samarakoon, 2023; Kumarasinghe et al., 2018). Moreover, the temperature was set to 70 ± 2°C for another 15 minutes to induce protein denaturation, accelerating structural unfolding and stimulating protein damage. Afterward, the test tubes were removed from the water bath, leaving the samples to dry at room temperature for 10 minutes, before isolating the precipitate.

Fourier Transform Infrared (FTIR) Spectroscopy

Three samples (100% taro leaf extract, diclofenac sodium, and distilled water) were subjected to FTIR Spectroscopy using a Perkin Elmer FT-IR Spectrometer Frontier with an infrared range of 4000 – 600 cm^{-1}. The reference method was an In-house Procedure (AL-TP-400 Fourier Transform Infrared Spectroscopy) with 20 scans per sample by the DOST-ITDI. The baseline correction was applied to the spectrum to improve its quality without distorting the band intensities in the final spectrum. An attenuated total reflectance technique was used, which allowed direct examination of solid or liquid samples without further preparation. It was done through total internal reflection, generating an evanescent wave penetrating the sample and providing valuable molecular information (Mettler-Toledo International Inc., 2023). Deconvolution was conducted afterwards to separate overlapping peaks for quantification of protein secondary structures.

Gravimetric Analysis

All groups were measured using gravimetric analysis, a test used to measure the weight of a sample. Each precipitate was separated from the remaining liquid after protein denaturation and placed in an analytical balance, evaluating protein denaturation inhibition of taro leaf extract and how much less it weighed compared to the control groups. The mass yielded was directly recorded per sample and organized per group for a surface level and complimentary analysis only.

Statistical Analysis and Data Interpretation

The organized and collected data were analyzed and interpreted through statistics. A statistician computed and determined if there were significant differences in the clinical application between the experimental taro leaf extract (12.5%, 25%, 50%, and 100%), positive control (diclofenac sodium), and negative control (distilled water). A one-way analysis of variance was employed to determine if there are significant differences in mass among the experimental, positive, and negative control groups, as it compares the means of three or more independent groups under a single experimental condition. Tukey's HSD post-hoc test was applied afterward to identify which treatment pairs (high-concentration vs. low-concentrations vs. positive control vs. negative control) differed significantly. It described whether observed mass changes subjected to different treatments were the cause of specific extract concentrations rather than random variation, ensuring the validity of the collected data and the interpretation of the results.

2.6 Ethical Considerations

Risks

Laboratory personnel supervised the researchers' experiments to ensure all procedures were performed safely and accordingly. It guarantees precise and accurate results. It also substantially minimizes risks to both the study materials and the researchers.

Proper waste disposal

The study used materials that were considered hazardous wastes, such as ethanol from taro leaves extraction. Through this, the materials prevent contamination, exposure, and contact with people. Additionally, the researchers were equipped with Personal Protective Equipment (PPE) throughout the experimentation to prevent injury through absorption, inhalation, or physical contact.

3.0 Results and Discussion

Figure 1 shows the stacked FTIR spectrum of egg albumen treated with 100% taro leaf extract (TLE), diclofenac sodium, and distilled water. The infrared values of each peak in the spectrum provide insights into the possible interactions among the different additives. Broad peaks of 100% TLE and diclofenac sodium treated egg albumen revealed IR values of 3297.22 cm^{-1} and 3267.92 cm^{-1} , linked to O-H stretching, confirming the presence of hydrogen bond interactions. Moreover, the findings of (Majaliwa et al., 2025) suggest that this indicates the strong intermolecular forces between the proteins of egg albumen and phytochemicals such as those found in 100% TLE, and compounds found in diclofenac sodium. Furthermore, the broad peak of the untreated egg albumen spectra with the IR value of 3305.37 cm^{-1} was linked to N–H/O–H stretching, suggesting hydrogen bonding interactions between the proteins found in egg albumen when undergoing stress factors such as applied heat during the protein denaturation procedure.

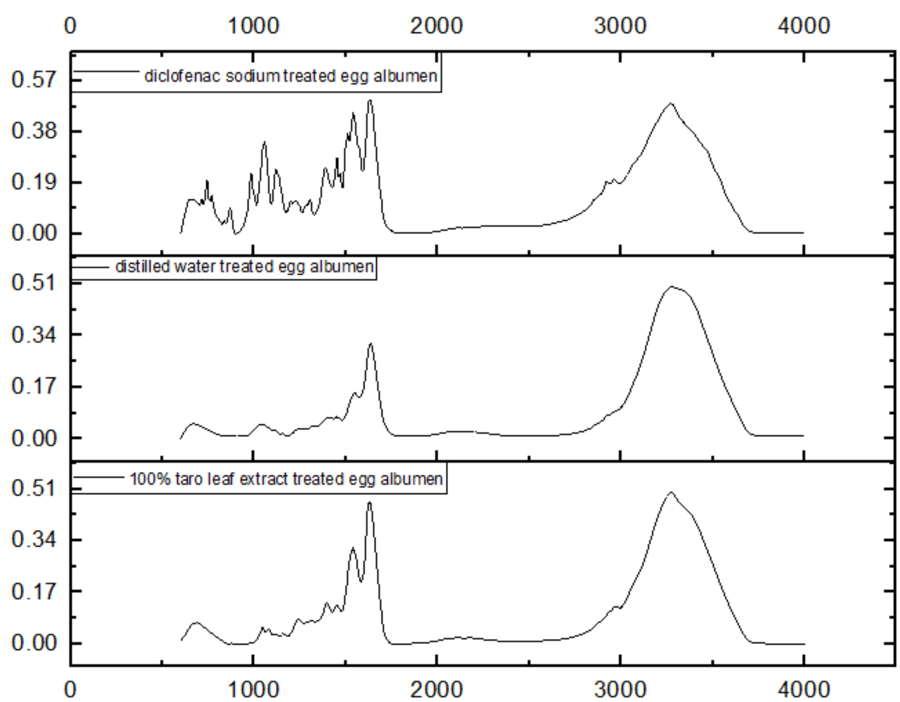

Figure 1. *FT-IR spectrum of samples treated with egg albumen*

Table 1 presents the assigned peaks found in the spectra and their corresponding functional groups for the different samples. Peaks between 2980-2900 cm^{-1} are present among samples, corresponding to C-H stretching vibrations linking to aliphatic side chains that contribute to the overall structure of egg albumen proteins

(Movasaghi et al., 2008). Absorptions that proved prominent in the 1740-1630 cm⁻¹ region correspond to the amide I band with C=O stretching, while those in the 1630-1509 cm⁻¹ region correspond to the amide II band with N-H bending and C-N stretching, both characteristic of peptide linkages in proteins as per Barth (2007). Moreover, additional absorptions recorded between the 1455-1350 cm⁻¹ and 1250-1000 cm⁻¹ linked to side chain and alkyl group vibrations, also making up the structure and function of egg albumen. Ether was identified with the 1055.45 cm⁻¹ peak recorded within the 1200-900 region for egg albumen with diclofenac sodium, possibly due to purification factors of its non-laboratory grade form (Kebebe et al., 2012). Egg albumen with 100% taro leaf extract and distilled water primarily share hydroxyl, alkane, and amide group signatures. However, these samples' absence of ether and alkene peaks reflects the absence of drug-related functional groups.

Table 1. *Peak Assignments in the Infrared Spectra of the Samples*

Standard Group	Frequencies (cm^{-1}) Egg albumen w/ 100% taro leaf extract	Egg albumen w/ diclofenac sodium	Egg albumen w/ distilled water	Compound	Bonds
3600-3200	3297.22	3267.92	3305.37	Hydroxyl	O-H Stretch
2980-2900	2964.28	2964.58	2967.85	Alkane	C-H Stretch
1740-1630	1630.45	1632.92	1635.85	Amide	C=O Stretch
1630-1509	1539.46	1541.58	1549.08	Amide	N-H Bend
	-	1509.57	-		N-C=O Stretch
1455-1350	1452.06	1451.94	1453.33	Amide	C-N Stretch
	1398.12	1389.56	1404.33		
1350-1290	-	1305.38	-	Alkene	=CH Stretch
1250-1000	1241.99	1200.42	1039.91	Alkane	C-H Bend
	1046.56	-	-		
1200-900	-	1122.11	-	Ether	C-O Stretch
	-	1055.45	-		
	-	984.72	-		
900-800	-	869.26	-	Alkane	C-H Bend
770-720	-	769.26	-	Alkane	C-H Bend
	-	744.46	-		
720-600	684.27	714.62	674.06	Amide	N-H Bend
	-	660.72	-		

Table 2 shows the different deconvolution results after analyzing the samples' peaks in the Amide I region. These parameters allowed the identification of secondary protein structures and the calculation of their area percentages using center gravity and area integration for each peak. In particular, a majority of protein structure maintained was seen with the substantial 77.62% area percentage of the α-helix in 100% concentration taro leaf extract treated egg albumen, contrasting with distilled water treated egg albumen that has a notably lower percentage, with a 22.38% area percentage of random coil.

Table 2. *Amide I Region Deconvolution Results of Experimental, Positive, and Negative Control Groups*

Sample	Peak	Center Gravity	Area Integration	Secondary Protein Structure	Area Percentage
Egg albumen with 100% taro leaf extract	1635	1628.20	2.23	β-sheet	22.38%
	1645	1650.17	7.75	α-helix	77.62%
Egg albumen with diclofenac sodium	1625	1624.65	17.64	β-sheet	60.23%
	1646	1646.31	11.65	random coil	39.77%
Egg albumen with distilled water	1635	1634.76	3.53	α-helix	38.22%
	1640	1640.43	1.96	β-sheet	21.22%
	1651	1650.56	3.75	α-helix	40.56%

A higher alpha helix content suggests a greater preservation of the native secondary protein structure commonly found in egg albumen, which is predominantly composed of α-helices. It showed similarity with diclofenac sodium; however, while no random coil peaks were detected, the β-sheet component was notably elevated compared to both experimental and negative control groups. The increase in β-sheet content suggests a tendency toward protein aggregation similar to β-sheet content-related aggregation in amyloid peptides discussed by Liu et al. (2018).

Table 3 compares the gravimetric analysis of groups to determine the protein denaturation inhibition of taro leaf extract compared to the control groups. Among the three samples in the experimental group, 100% concentration consistently showed the highest mass compared to 50%, 25%, and 12.5% concentrations. This trend suggests that higher concentrations of the extract increased the mass of the egg albumen, promoting properties that increase protein aggregation and coagulation rather than anti-inflammatory properties. Meanwhile, the lower concentrations showed significantly lower masses, indicating lower protein aggregation. This increase in mass can be associated with aggregation and clumping activity, supporting the study of Panganiban et al. (2024) in exploring taro leaf extract as a clot activator.

Table 3. *Gravimetric Analysis of Experimental, Positive, and Negative Control Groups*

Set-up	Group	Trial 1 (*g*)	Trial 2 (*g*)	Trial 3 (*g*)
Experimental	12.5%	2.61	2.53	2.99
	25%	2.60	2.90	2.75
	50%	2.88	2.70	2.70
	100%	3.95	3.42	4.18
Positive Control	Diclofenac Sodium	4.48	5.17	4.10
Negative Control	Distilled Water	3.03	3.42	3.76

Studies by Nwaogwugwu (2020) and Sjamsudin et al. (2021) have incorporated taro leaves in diabetes management and wound healing through wound care products and dressings, further highlighting its role as a coagulative agent. However, also found in the study of Panganiban et al. (2024) is the hemolysis of blood caused by the 100% concentration, suggesting that very high concentrations can overshadow its supposed effect, causing dosage toxicity through oxidative stress, especially with taro leaves' high oxalate level. Further studies are needed to define the role of dosage in the effect of taro leaf extract on proteins, particularly in egg albumen.

Moreover, Table 4.1 presents the one-way ANOVA analysis between different treatment groups on protein denaturation. The between-groups variation (SS = 8.674, df = 5, MS = 1.735) is significantly larger than the within-groups variation (SS = 1.344, df = 12, MS = 0.112), which results in an F-value of 15.49. The results indicate significant differences between groups since the p-value 7.11E-05 is less than 0.05. Moreover, the F-value exceeds the F crit = 3.11, denoting at least one group that differs significantly. With the significance between-group variance, findings suggest that the concentrations of taro leaf extract with the positive and negative control groups yielded significant differences in their effects on the protein denaturation of egg albumen.

Table 4.1. *ANOVA Analysis on Weight between Groups*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	8.674	5	1.735	15.49	7.11E-05	3.11
Within Groups	1.344	12	0.112			
Total	10.017	17				

Table 4.2 presents the performed Tukey's HSD post-hoc Test, evaluating differences between concentrations of taro leaf extract, positive and negative controls, and their effects on protein denaturation. 100% concentration significantly differs from 12.5% (p = 0.01), 25% (p = 0.02), and 50% (p = 0.02), suggesting its high concentration played a role in its distinct structural impact. Although 100% concentration did not significantly differ from the negative control (p = 0.58), the variability in aggregation extent or other non-specific mass changes could account for its final result. Additionally, the findings of Novak et al. (2023) concluded that a protein's secondary structure, particularly the a-helix, can be maintained even in fibril formation as seen in the high a-helix content in the deconvolution of its amide I region. Moreover, TLE's high concentration of calcium oxalate crystals could explain why aggregation might have led to the yielded mass, especially in increased concentrations (Panganiban et al, 2024; Shal et al, 2022).

Table 4.2. *Summary of Independent Samples T-test Analysis of Between Groups*

Pairwise comparison		t Statistic	P-value	Significance
12.5%	25%	0.18	0.89999	insignificant
	50%	0.23	0.89999	insignificant
	100%	5.88	0.01299	p < .05
	Positive control	9.68	0.00101	p < .01
	Negative control	3.57	0.19203	insignificant
25%	50%	0.05	0.89999	insignificant

	100%	5.70	0.01610	p < .05
	Positive control	9.50	0.00101	p < .01
	Negative control	3.39	0.23202	insignificant
50%	100%	5.65	0.01712	p < .05
	Positive control	9.45	0.00101	p < .01
	Negative control	3.33	0.24447	insignificant
100%	Positive control	3.80	0.14944	insignificant
	Negative control	2.31	0.58406	insignificant
Positive control	Negative control	6.11	0.00986	p < .01

In contrast, diclofenac sodium had both a significant difference with all groups, and a notably high b-sheet content leading towards distinct aggregation and change in the egg albumen's secondary protein structure. Although known for its anti-inflammatory effect, diclofenac sodium's impurity in tablet form could also account for its inability to preserve native protein structure; however, since no pre-test was conducted, factors such as sample condition before protein denaturation requires setup modification in further studies before being ruled out.

4.0 Conclusion

Structural differences were observed through the shifts in Amide I bands of the 100% taro leaf extract-treated egg albumen, suggesting the presence of its bioactive compounds through strong hydrogen bonding interactions, indicating aggressive protein aggregation. It contrasted with the positive control (diclofenac sodium) role in protein stabilization, suggesting that the extract promotes coagulation rather than preventing protein misfolding. Additionally, multiple absorptions were recorded in carbon group stretching and bending, side chains, and alkyl group vibrations, confirming the presence of some preserved native protein structures in all samples. Ether was identified only in diclofenac sodium's absorption values, potentially accounted for by an ingredient in its tablet coating known as polyethylene glycol ether, a binding agent. The deconvolution results of the Amide I region of the experimental, positive, and negative control groups reveal the secondary structural differences, particularly in 100% concentration treated egg albumen, yielding the highest α-helix content. It maintained most of the native secondary structures of egg albumen as distinct differences were found in the absence of random coils compared to distilled water-treated egg albumen, indicating the protein stabilizing capability of taro leaf extract. Similarly, diclofenac sodium has no presence of random coil; despite its anti-inflammatory capability, the sample has notably high β-sheet content, leading to aggregation instead of other groups with lower percentages. The gravimetric analysis further supported this claim, revealing the highest mass from diclofenac sodium, possibly affected by the impurities of its tablet form with other binding ingredients and high dosages as the researchers used its pure form. Moreover, the 100% concentration also revealed a significantly higher mass than lower concentrations. Despite the aggregation observed with the increase of mass and insignificant difference with the negative control group, deconvolution results imply the preserved native secondary structure of the egg albumen as α-helices tend to maintain their structure even during fibril or aggregate formation. This may be attributed to other components in taro leaf extract, such as its high oxalate content and clot-activating properties, which promote coagulation despite preserving secondary structure stability, prompting further studies to confirm these findings and their respective factors.

5.0 Contribution of Authors

All authors have approved the work. Sebastian Panganiban served as guidance in publication proceedings and Marcelino Gigantone as research adviser of the remaining authors.

6.0 Funding

No funding was made for this research.

7.0 Conflict of Interest

The authors declare that they have no conflict of interest.

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