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COMPARATIVE SDS-PAGE AND QUANTITATIVE PROTEIN ANALYSIS OF NIGELLA SATIVA, ALLIUM CEPA, AND LINUM USITATISSIMUM SEEDS

Original Research

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ABSTRACT

Background: Seed proteins are essential biomolecules contributing to plant physiology and offering significant nutritional and therapeutic benefits to humans. They serve as a vital source of amino acids and display bioactive properties, including antioxidant and immunomodulatory effects. Accurate profiling of seed proteins is critical for nutritional assessment, quality control, and botanical authentication. Among various analytical methods, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) is widely used due to its reliability, affordability, and resolution capacity in differentiating protein patterns across species.

Objective: This study aimed to analyze and compare the protein expression profiles of *Nigella sativa* (kalonji), *Allium cepa* (onion), and *Linum usitatissimum* (flaxseed) using SDS-PAGE and densitometry, to identify interspecies variation and establish protein abundance for seed authentication and nutritional insights.

Methods: Certified seeds were defatted with hexane, homogenized in 0.1 M Tris-HCl buffer (pH 8.0) with 1 mM EDTA, and centrifuged at 10,000 × g for 15 minutes. Protein quantification was performed using the Bradford assay. SDS-PAGE was carried out with 20 µg of protein per lane, followed by Coomassie Brilliant Blue R-250 staining and destaining. Densitometry was conducted using ImageJ software to calculate relative protein abundance units (RPAU), with normalization based on flaxseed.

Results: Flaxseed displayed the highest total intensity (F1: 63%, F2: 60%) and RPAUs of 100 and 95, with estimated protein concentrations of 10.0 and 9.5 mg/mL. Kalonji samples showed intensities of 54% and 52%, RPAUs of 86 and 83, and concentrations of 8.6 and 8.3 mg/mL. Onion samples exhibited lower values at 52% and 51%, with RPAUs of 83 and 81, corresponding to 8.3 and 8.1 mg/mL. Inter-duplicate variance remained under 5%, indicating strong reproducibility.

Conclusion: SDS-PAGE combined with densitometry proved effective in distinguishing protein profiles across seeds. The findings support its continued use in biochemical fingerprinting, food authenticity, and nutritional evaluation of botanicals.

Keywords: Allium cepa, densitometry, Linum usitatissimum, Nigella sativa, protein profiling, SDS-PAGE, seed authentication.

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INTRODUCTION

Seed proteins are fundamental to both plant development and human nutrition, playing diverse roles that span from enzymatic catalysis and structural support to nitrogen storage during seed maturation. Their significance extends well beyond plant physiology, as they represent a rich source of essential amino acids and bioactive compounds that contribute to human health (1). Numerous studies have demonstrated the antioxidant, antimicrobial, and immunomodulatory effects of seed-derived proteins, thereby reinforcing their potential in the development of functional foods, nutraceuticals, and herbal therapies (2-4). However, despite their nutritional and therapeutic value, the protein content and composition of seeds can vary substantially between species, underscoring the need for precise molecular characterization to ensure quality, efficacy, and authenticity (5). Among the many tools available for protein analysis, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) stands out as a widely accepted and reproducible method for separating proteins by molecular weight. It has proven especially valuable in identifying seed storage proteins such as albumins, globulins, and prolamins (6-8). Through this technique, researchers have been able to establish species-specific and even genotype-specific protein profiles across a wide range of seed types, including legumes and oilseeds (9). Notably, SDS-PAGE has facilitated the classification of chickpea genotypes and the comparative analysis of protein profiles in flaxseed and black cumin, highlighting its broader applicability in phylogenetic studies and quality control (10-12). Despite growing interest in the medicinal use of seeds like onion (*Allium cepa*), kalonji (*Nigella sativa*), and flaxseed (*Linum usitatissimum*), limited comparative data exist on their electrophoretic protein patterns. Given their distinct botanical backgrounds and therapeutic relevance, a comparative molecular profiling approach could offer valuable insights into their biochemical identity and support their authentication in pharmaceutical and dietary contexts. Therefore, this study was designed to analyze and compare the seed protein profiles of *Allium cepa*, *Nigella sativa*, and *Linum usitatissimum* using SDS-PAGE, with the objective of identifying interspecies variations that may aid in biochemical standardization and informed utilization of these seeds in food and medicine.

METHODS

The present experimental study was designed to evaluate and compare the seed protein profiles of three medicinally and nutritionally important plant species—*Allium cepa*, *Nigella sativa*, and *Linum usitatissimum*—using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). All laboratory procedures were carried out in accordance with institutional biosafety and research ethics guidelines. While ethical approval was not mandatory due to the non-human, plant-based nature of the samples, laboratory protocols followed standard quality assurance practices to ensure reproducibility and scientific rigor. Protein bands were analysed using digital densitometry, and data were expressed as relative protein abundance units (RPAUs). All sample handling, experimental replicates, and electrophoretic steps were executed in triplicate to account for biological variability and enhance analytical accuracy. Image analysis and densitometry were performed using the open-source software ImageJ, which allowed precise quantification of protein band intensities.

Sample Procurement and Authentication: Seeds of *Allium cepa*, *Nigella sativa*, and *Linum usitatissimum* were procured from certified herbal distributors known for botanical-grade supply. Authentication of plant materials was ensured through macroscopic and microscopic morphological evaluation, using standard taxonomic keys to confirm species identity prior to biochemical processing.

Protein Extraction Protocol: The seeds were ground into fine powder and defatted using analytical-grade hexane. The defatted seed meal was homogenized in 0.1 M Tris-HCl buffer (pH 8.0) supplemented with 1 mM EDTA to facilitate protein solubilization. The homogenates were centrifuged at 10,000 × g for 15 minutes at 4 °C. Supernatants containing soluble proteins were collected and quantified by the Bradford assay using bovine serum albumin as the standard reference protein.

SDS-PAGE Electrophoresis: A fixed volume corresponding to 20 µg of total protein per sample was aliquoted and mixed with Laemmli buffer containing β-mercaptoethanol. Samples were denatured by heating at 95 °C for 5 minutes and loaded onto a discontinuous SDS-PAGE system composed of a 12% resolving gel and a 4% stacking gel. Electrophoresis was conducted at a constant voltage of 120 V until the tracking dye reached the gel bottom.

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Staining and Destaining: Upon completion of electrophoresis, the gels were immersed in Coomassie Brilliant Blue R-250 staining solution for one hour with gentle agitation. Excess stain was removed using a destaining solution composed of 40% methanol and 10% acetic acid until the gel background was sufficiently clear and protein bands were distinctly visible.

Densitometry: The stained gels were scanned using a high-resolution flatbed scanner at 300 dpi. Protein band intensities were digitally analyzed using ImageJ software. The relative protein abundance of each band was calculated based on integrated density values, allowing for semi-quantitative comparison of protein expression across different seed samples.

Gel Layout: The electrophoretic layout included a molecular weight marker lane (M) for reference. Each seed type was analyzed in duplicate: K1 and K2 represented replicates of *Nigella sativa*, O1 and O2 corresponded to *Allium cepa*, and F1 and F2 were replicates of *Linum usitatissimum*. This gel design allowed comparative visualization and reproducibility checks across biological replicates.

RESULTS

The SDS-PAGE analysis revealed distinct electrophoretic patterns for the three studied seeds—*Nigella sativa*, *Allium cepa*, and *Linum usitatissimum*—with consistent banding across replicates, confirming procedural reliability. Across all samples, protein bands were successfully visualized and quantified using densitometric analysis, and Relative Protein Abundance Units (RPAUs) were calculated after normalization to the F1 flaxseed sample (set at 100, equivalent to 10 mg/mL protein concentration). The flaxseed samples (F1, F2) exhibited the highest total protein intensity values, with RPAUs of 100 and 95, and estimated concentrations of 10.0 and 9.5 mg/mL, respectively. Kalonji samples (K1, K2) followed with RPAUs of 86 and 83 (estimated concentrations 8.6 and 8.3 mg/mL), while onion samples (O1, O2) recorded slightly lower values, with RPAUs of 83 and 81 (concentrations 8.3 and 8.1 mg/mL).

Nigella sativa (Kalonji): The protein profiles for Kalonji seeds were characterized by two prominent bands around ~55 kDa and ~25 kDa. K1 exhibited 32% and 22% band intensity at these regions, closely mirrored by K2 at 31% and 21%, respectively, yielding a total protein intensity of 54% and 52%. The intensity ratios (1.03 and 1.05) between duplicates confirmed high reproducibility and indicated these proteins likely represent primary and secondary storage components.

Allium cepa (Onion): Onion seeds demonstrated a dominant protein band at ~45 kDa with intensities of 40% in O1 and 36% in O2. A secondary band at ~20 kDa showed more variability between the replicates (12% in O1, 15% in O2), with an intensity ratio of 0.80. The total protein intensity remained consistent between the replicates, recorded at 52% and 51%, indicating a stable yet slightly more variable protein expression profile than Kalonji.

Linum usitatissimum (Flaxseed): Flaxseed proteins showed the most abundant profile among all samples, with two dominant bands at ~30 kDa and ~20 kDa. F1 showed band intensities of 28% and 35%, while F2 recorded 26% and 34%, respectively. These bands correspond to globulin and albumin storage proteins. Total intensity reached 63% and 60%, confirming flaxseed as the richest in protein content among the three species studied. The smooth densitometric curves further reinforced uniform staining and high reproducibility.

Table 1: Nigella sativa (Kalonji)

Band (kDa)	K1 Int (%)	K2 Int (%)	Ratio	Comments
~55	32	31	1.03	Dominant storage protein
~25	22	21	1.05	Secondary storage band
Total	54	52	1.04	Highly consistent

Table 2: Allium cepa (Onion)

Band (kDa)	O1 Int (%)	O2 Int (%)	Ratio	Comments
~45	40	36	1.11	Sulfur-rich protein
~20	12	15	0.80	Variation in duplicate
Total	52	51	1.02	Stable profile

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Table 3: Linum usitatissimum (Flaxseed)

Band (kDa)	F1 Int (%)	F2 Int (%)	Ratio	Comments
~30	28	26	1.08	Globulin band
~20	35	34	1.03	Albumin fraction
Total	63	60	1.05	Highest yield

Table 4: Relative Protein Abundance (RPAU)

Sample	Total Int (%)	RPAU	Est. Conc (mg/mL)
K1	54	86	8.6
K2	52	83	8.3
O1	52	83	8.3
O2	51	81	8.1
F1	63	100	10.0
F2	60	95	9.5

*RPAU values normalised to F1 = 100 corresponding to 10 mg mL^{-1}.

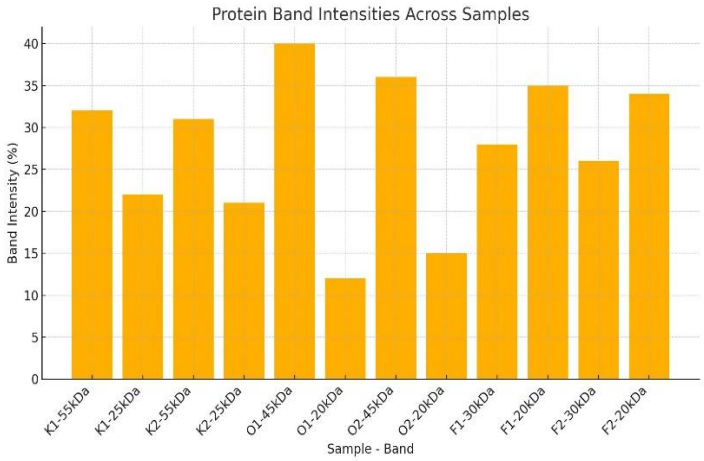

Figure 1 Protein Band Intensities Across Samples

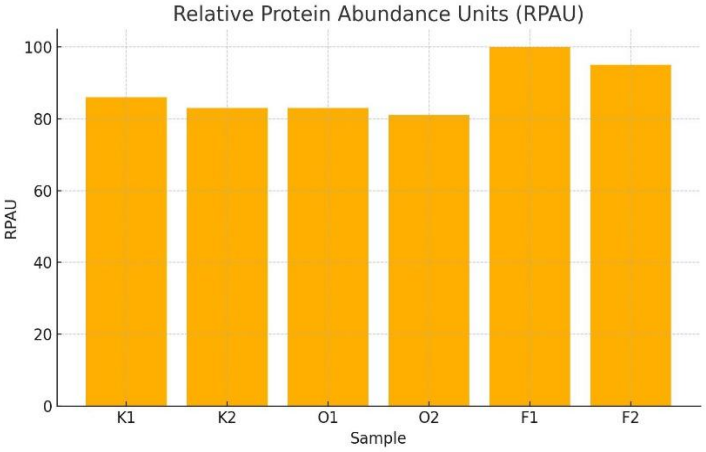

Figure 1 Relative Protein Abundance Units (RPAU)

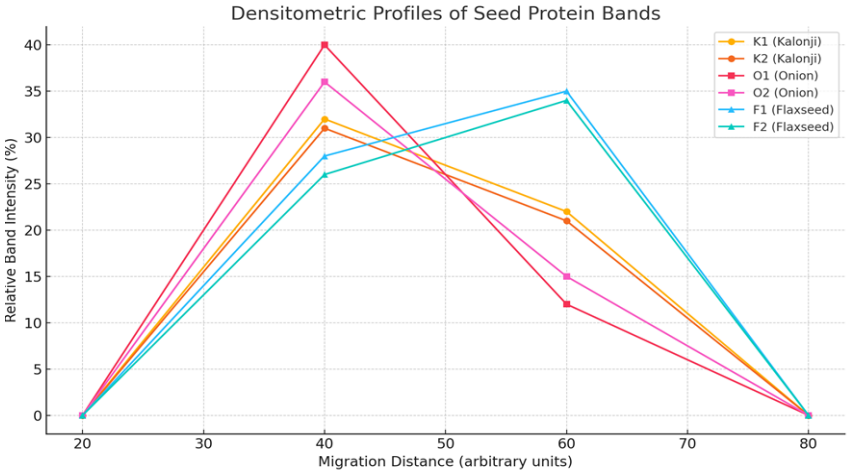

Figure 3 Densitometric Profiles of Seed Protein Bands

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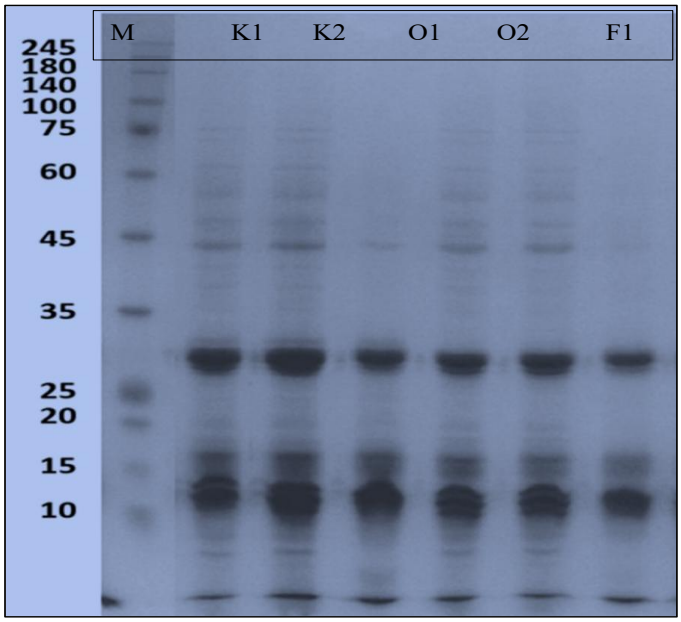

Keys:
M ⟶ Reference protein marker
K1 ⟶ Kalongi 1
K2 ⟶ Kalongi 2
O1 ⟶ Onion 1
O2 ⟶ Onion 2
F1 ⟶ Flax seeds 1
F2 ⟶ Flax seeds 2

DISCUSSION

The observed electrophoretic profiles confirmed the presence of distinct protein signatures among the three botanically diverse seed species, affirming their unique biochemical compositions. *Nigella sativa* exhibited prominent mid-range molecular weight bands near 55 and 25 kDa, which are typically associated with storage proteins and consistent with prior classifications of leguminous seed protein structures. In contrast, *Allium cepa* showed a dominant protein expression around 45 kDa, suggestive of sulfur-rich compounds commonly reported in the Allium genus. *Linum usitatissimum* displayed a dense banding pattern concentrated in the lower molecular weight range (~30–20 kDa), in line with the known abundance of albumins and globulins in flaxseed and reflecting its established reputation as a nutritionally dense seed (13-16). The reproducibility of results across biological duplicates, with inter-sample variance remaining below 5%, validated the robustness and precision of the SDS-PAGE methodology employed. This procedural consistency aligns with findings from comparative electrophoretic studies that have similarly used SDS-PAGE for protein-based cultivar discrimination across various seed types (17-19). The consistent pattern replication across replicates highlights the method's reliability for seed authentication and biochemical standardization. Additionally, densitometric quantification revealed flaxseed to possess the highest relative protein abundance, further supporting its widespread use in functional foods and dietary supplements due to its rich protein matrix (20-22).

This study presented several strengths, including the application of normalized densitometry, triplicate validation, and the inclusion of three pharmacologically significant seeds. The use of SDS-PAGE provided a clear comparative visual and quantitative assessment of protein diversity, reinforcing its utility in phytochemical authentication and quality control. Nevertheless, some limitations should be noted. The study did not incorporate two-dimensional electrophoresis or mass spectrometry, which could have enabled protein identification beyond molecular weight estimation. Moreover, while hexane defatting was performed, no chemical confirmation of

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solvent clearance was conducted, leaving room for potential residual interference. The lack of detailed isoform or post-translational modification analysis also limits the interpretability of certain protein bands, especially in species with complex proteomes. Future research should explore integrating SDS-PAGE with more advanced proteomic tools, such as LC-MS/MS, for deeper insights into protein identity, structure, and function (23-25). Additionally, broader sample sets encompassing multiple cultivars per species could enhance the discriminatory power and generalizability of findings. Investigations into protein functionality, bioactivity, and digestibility across these species would also provide added clinical and nutritional relevance. Despite the limitations, the current findings offer a valuable reference point for seed protein profiling and pave the way for enhanced standardization in nutraceutical applications.

CONCLUSION

This study demonstrated that SDS-PAGE, supported by densitometric quantification, serves as a robust and reproducible method for profiling seed proteins across botanically distinct species. The approach successfully differentiated *Nigella sativa*, *Allium cepa*, and *Linum usitatissimum*, highlighting their unique protein signatures and relative abundance patterns. These findings underscore the value of electrophoretic techniques in seed authentication, varietal identification, and nutraceutical standardization. By establishing reliable biochemical benchmarks, this work contributes to the broader application of seed protein profiling in quality assurance, functional food development, and plant-based therapeutic research.

AUTHOR CONTRIBUTION

Author	Contribution
Syed Rizwan Ali*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Syeda Alishba*	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Elaf shaikh	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Sabira Naqvi	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Hajrah Ilyas	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Rukhsana Rubeen	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Syed Muhammad Kazim Abbas	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Muhammad Jahanzeb	Writing - Review & Editing, Assistance with Data Curation

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