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Biochemical characterization and profiling of major seed storage proteins in selected medicinal plant found in Sindh Pakistan

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Abstract: *In this study investigated the composition and biochemical properties of seed storage proteins in important medicinal plants, including the seeds of*

Syzygium cumini L., Lawsonia inermis L., Calotrpis procera. R Bro, Ricinus communis L., Linum usitatissimum L., Brassica juncea L Czern., Withania coagulans Dun, and Cucumis melo var. agrestis Naudin. Protein extraction from seed flour was carried out using sequential steps of extraction: delipidation (removal of oil), water (albumin), 5.0 M NaCl (globulin), 70% ethanol (prolamin), and 150.2M sodium phosphate buffer, pH 8.0 (glutelin). Quantification was performed using the dye binding method of Bradford which showed substantial differences in their concentrations and total yields. Comparative analysis of seed storage proteins showed different dominance patterns among the plants studied. The highest levels of albumin were found in Brassica juncea L Czern. (50.40%), which suggests a strong water-soluble protein fraction, the highest globulin content was found in Ricinus communis (67.11%), suggesting a high salt-soluble protein fraction, the highest prolamin content was found in Withania coagulans (55.93%), suggesting a strong alcohol-soluble protein fraction, and the highest glutelin was found in Syzygium cumini (15.12%).

Keywords: *Seed storage proteins (SSPs) Extraction, Protein estimation, Medicinal plants*

INTRODUCTION

During seed germination and the early stages of seedling growth, seed storage proteins serve as important stores of nitrogen and amino acids. These proteins are classified based on their solubility and structural properties into albumins, globulins, prolamins, and glutelins. In addition to their physiological role in plants, seed storage proteins have become more interesting to study due to their nutritional value and bioactive activities, such as antibacterial, antioxidant, and medicinal properties (Yang et al., 2023; Zeng et al., 2024),

Recent studies have also emphasized the importance of medicinal plant seeds as a source of bioactive peptides and functional proteins with pharmacological applications (Latif & Nawaz, 2025). The seeds of *Syzygium cumini L.* rich in albumins and globulins and are well known for their antioxidant and antidiabetic activities, which are attributed to a variety of bioactive chemicals and protein fractions (Latif & Nawaz, 2025). The presence of high levels of storage proteins, especially albumins, and its hepatoprotective and antibacterial properties make *Lawsonia inermis L.* an important plant in traditional medicine (Kumar et al., 2024). A number of protein components, including defense-related and enzymatic proteins, have anti-inflammatory and antibacterial properties in the seeds of *Calotropis procera R. Br.* (Tiwari et al., 2022). *Ricinus communis L.* seeds contain both toxic proteins such as ricin and storage proteins such as globulins, but the seeds can be used medicinally if properly prepared (Yang et al., 2023). The seeds of *Linum usitatissimum L.* contain globulins and albumins and are used medicinally for their cardioprotective, antioxidant, and anticancer activities, in part due to the composition of their proteins and related metabolites (Zeng et al., 2024). Due to their intricate biochemical composition, *Brassica juncea L Czern.,* seeds contain antibacterial and chemoprotective compounds and high levels of globulins (Yang et al., 2023). Research on storage protein albumin, globulin, prolamin, and glutelin. of Apiaceae Family medicinal plant, seeds are valuable, nutrient sources of seed proteins ,these seeds are utilized in home remedies to manage gastrointestinal issues such as indigestion, flatulence, and colic pain, while also acting as flavoring, aromatic, and preservative agents in food supplies (S.K. Khanzada et al. 2021), Bioactive proteins and secondary metabolites like withanolides are reported to be responsible for the antidiabetic and immunomodulatory properties of *Withania coagulans Dun.* seeds (Latif et al , 2025). Storage proteins are also important from a nutritional standpoint, and seeds of *Cucumis melo var. agrestis Naudin.* provide antioxidant and anti-inflammatory properties for traditional therapeutic use (Kumar et al., 2024). They have been investigated as a source of food and pharmaceuticals due to their biochemical composition, nutritional importance, and

medicinal properties, including medicinal plant seed storage proteins (M. Kumar et al., 2022). In human metabolism, seed storage proteins exhibit a wide range of activities, and albumins and globulins are abundant in angiospermic plants, including monocots and dicots, such as cereals, ferns, and palms (Templeman et al., 1987). Glutelins, on the other hand, are resistant to pepsin digestion and thus less digestible by humans. The biochemical characterization of (SSPs) in medicinal plants is important for understanding their nutritional value and therapeutic applications in the food and pharmaceutical industries. SSPs are simple biomolecules that are necessary for the growth, development, and reproduction of plants. (López-Guzmán et al., 2025). In this study investigated the biochemical properties and protein profiles of seed storage proteins in eight medicinally important plant species of various families.

MATERIAL AND METHODS

Material: Seeds of eight medicinally important plants belonging to the different plant families: Syzygium *cumini L.,* (Myrtaceae), *Lawsonia inermis L.,* (Lythraceae), *Calotrpis procera. R Bro* (Asclepiadaceae), *Ricinus communis L.,* (Euphorbiaceae), *Linum usitatissimum L.,* (Linaceae) *Brassica juncea L Czern.,* (Brassicaceae), *Withania coagulans Dun* (Solanaceae), and *Cucumis melo var. agrestis Naudin.* (Cucurbitaceae). were collected from three different localities, Nawabshah, Hyderabad, Jamshoro University of Sindh Jamshoro for conducted to investigate the composition, biochemical properties of seed storage proteins in important medicinal plants.

Seed storage protein extraction: The seed were ground with mortar pistil**,** the flour was delipidated by solvent extraction by stirring with pure hexane (1:10 w/v) for 30 min, and this procedure repeated three times at room temperature. Using the microextraction method (Sammour et al., 1999), the sample was air dried and kept under hood up to twenty-four hours in alcohol, water, high salt, and in alkaline buffer conditions. Finally, the seed was

extracted in triplicate in Eppendorf tubes with 500 micrograms (i) deionized water (ii) 145.0M NaCl (iii) 70% ethanol and (iv) 0.2 M, Na_3PO_4 buffer, pH8.0, to extract the four major seed storage proteins (prolamin, albumin, glutelin, and globulin), each at 500 micrograms, for 20 min at 350 rpm/25°C using Thermomixer comfort (Eppendorf, Germany). This extraction was performed at 14000 rpm for 15 min at 4°C (Biofuge Primo R Heraeus, Japan) and repeated twice to remove residual protein from each fraction. The supernatants of each fraction were pooled and stored at -20°C until protein profiling. The total protein from the entire extraction was measured using the modified color binding assay (Bradford, 1976) with bovine serum albumin as a standard. Protein quantitation was performed in triplicate using a microplate reader (Sunrise Tecan, Austria).

Results and Discussion

The seed storage protein content of eight medicinal plant species from Sindh, Pakistan is presented in Table 1. The results show noticeable variation among the species in the four main protein fractions, namely albumin, globulin, prolamin, and glutelin. In this study investigated the biochemical properties and protein profiles of seed storage proteins in eight medicinally important plant species of various families. *S.cumini L., L. inermis L.,* C. *procera. R Bro*, *R. communis L., L. usitatissimum L., B. juncea L Czern., W. coagulans Dun*, and *C.melo var. agrestis Naudin.*.These differences indicate that each species is composed of a different protein set, which could make it more or less nutritious and play different biological roles. Albumin showed a wide range of values (22.88 to 50.40), with *B. juncea L Czern* having the highest content (50.40), followed by *C. procera. R Bro*, (44.40), *L. inermis L.,* (41.86), *L. usitatissimum L.,* (40.14), and *W. coagulans Dun* (31.40), and the lowest in *R. communis L* (22.88). This high albumin proportion in *B. juncea L Czern* indicates it may be a good source of water-soluble proteins with high digestibility. Globulin values varied considerably, from 1.70% in *L. inermis L.* to 67.11% in *R. communis L.*,with *C. procera. R Bro*, (36.02%) and *L. usitatissimum L.,* (34.82%) also exhibiting significant

globulin levels. In a previous study, 43.25% protein recovery and 17.2% extraction efficiency was reported for *L. usitatissimum L.,* protein extracted at pH 12 (Sharma et al., 2022). However, the protein fractions differed and the major seed storage proteins were albumin (40.14%), globulin (34.82%), prolamin (22.38%), and glutelin (11.57%). *L. inermis L.,* (1.70%) and *W. coagulans Dun* (4.29%) contained the lowest amounts. These differences represent species-specific storage strategies and can impact seed nutritional quality and industrial applications. Prolamin was the major protein fraction in many species, *W. coagulans Dun* (55.00%), *L. inermis L.,* (53.32%), and *S.cumini L* (48.00%) followed by Cucumis melo var. agrestis (39.80%), *B. juncea L Czern* (26.56%), *R. communis L* (5.16%), and *C. procera. R Bro,* (7.75%). The high prolamin content in certain species suggests potential functional roles in seed physiology and possible applications in food and pharmaceutical industries, although prolamins are generally less nutritionally balanced due to low lysine content. Glutelin content ranged from 3.10% to 15.12%, with *S.cumini L* (15.12%) and *C.melo var. agrestis Naudin* (14.42%) showing the highest levels. Moderate amounts were observed in *C. procera. R Bro,* (11.81%) and *L. usitatissimum L.,* (11.57%), whereas *L. inermis L.,* (3.10%) and *B. juncea L Czern* (3.43%). The distribution of these proteins indicates that albumin and prolamin are the major proteins in most species, and that globulin is the major protein in *R. communis L.,* (SSPs) storage protein composition is highlighted by the observed variation in protein fractions, which may be impacted by genetic determinants, ecological adaptability, and seed functional needs.The extent to which the proportions of these proteins differ between species may have significant nutritional quality and industrial applications.

Table 1. Comparison of the concentrations and total percent yield of the major seed storage proteins from *Syzygium cumini L., Lawsonia inermis L. Calotrpis procera.R Bro Ricinus*

communisL., Linum usitatissimum L., Brassica juncea L Czern., Withania coagulans Dun, and Cucumis melo var. agrestis Naudin. seeds found in Sindh, Pakistan.

S.NO.	Plant Name	FamilyName	Albumin mg/g	%yield	Globulin * # mg/g	%yield	Prolamin * # mg/g	%yield	Glutelin * # mg/g	%yield
1.	*Syzygium cumini L.*	Myrtaceae	0.83	30.53	0.19	7.00	1.30	48.00	0.40	15.12
2.	*Lawsonia inermis L.*	Lythraceaea	0.61	41.86	0.02	1.70	0.78	53.32	0.04	3.10
3.	*Calotrpis procera.R Bro*	Asclepiadaceace	0.13	44.40	0.10	36.02	0.02	7.75	0.03	11.81
4.	*Ricinus communis L.*	Euphorbiaceae	1.86	22.88	5.47	67.11	0.42	5.16	0.39	4.81
5.	*Linum usitatissimum L.*	Linaceae	1.34	40.14	1.17	34.82	0.75	22.38	0.38	11.57
6.	*Brassica juncea L Czern.*	Brassicaceae	0.76	50.40	0.29	19.60	0.40	26.56	0.05	3.43
7.	*Withania coagulance Dun.*	Solanaceae	0.08	31.44	0.01	4.29	0.15	55.00	0.02	9.32

8.	*Cucumis melo L.var. agrestis Naudin.*	Cucurbitaceae	0.03	30.77	0.01	15.00	0.05	39.80	0.01	14.43

* Concentrations in mg/g of seed flour. Values are mean of three independent extractions.

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% yield of a particular protein in total protein contents of seed flour.

Table 2: Seed storage protein (SSPs) composition, functional importance, and medicinal significance of selected medicinal plants found in Sindh Pakistan

Medicinal plants With family name	Importance of protein	Medicinal significance
Syzygium cumini (Myrtaceae)	Provide essential amino acids and support antioxidant defense mechanisms in human metabolism	Antidiabetic, anti-inflammatory, antioxidant
Lawsonia inermis (Lythraceae)	Easily digestible proteins (albumins) enhance nutrient absorption and immune support	Antimicrobial, antifungal, hepatoprotective
Calotropis procera (Asclepiadaceae)	Defense-related proteins may exhibit bioactive and therapeutic properties in small doses	Anti-inflammatory, analgesic
Ricinus communis (Euphorbiaceae)	Storage proteins include potent bioactive molecules; require detoxification for safe use	Laxative, antimicrobial, anti-inflammatory
Linum usitatissimum	High-quality proteins contributes to	Cardioprotective, antioxidant,

(Linaceae)	cardiovascular health and metabolic regulation	anticancer
Brassica juncea (Brassicaceae)	Rich in functional proteins (e.g., cruciferin) supporting detoxification and cellular protection	Antimicrobial, anti-inflammatory chemoprotective
Withania coagulans (Solanaceae)	Bioactive proteins and peptides aid in immune modulation and glucose regulation	Antidiabetic, hepatoprotective, immunomodulatory
Cucumis melo var. *agrestis* (Cucurbitaceae)	Nutritionally important proteins contribute to hydration balance and metabolic support	Antioxidant, anti-inflammatory, diuretic
Cucumis melo L.var. agrestis *Naudin.*	Nutritionally important proteins contribute to hydration balance and metabolic support	Antioxidant, anti-inflammatory, diuretic

Table 2 showed these medicinal plants are very valuable for human nutrition and therapeutic applications, seeds contain four types of protein (SSPs) that provide a balance of rapidly digestible proteins (albumins) and slow release, long-lasting proteins (globulins, prolamins, glutelins) to meet immediate metabolic needs, long-term amino acid supply, and delivery of bioactive compounds, which are directly linked to the observed antioxidant, antidiabetic, immunomodulatory, and cardioprotective activities of these plants. *L. usitatissimum L.,* and *L. inermis L.* seeds are rich sources of albumin and globulin and, therefore, are especially important for human nutrition and therapeutic use.

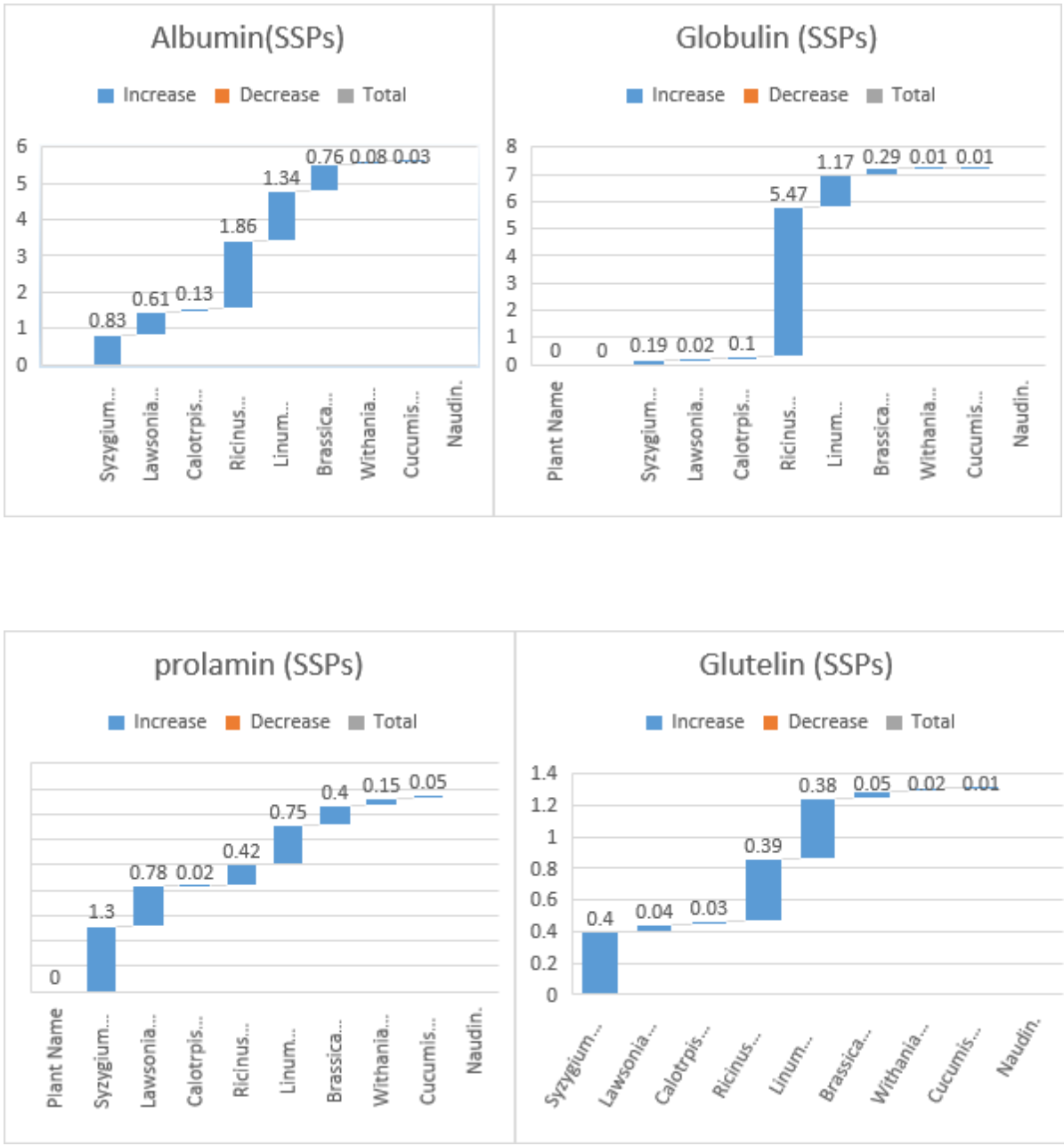

Fig. 1. Graphical representation of the four major seed storage protein (SSPs)(Albumin, Globulin, Prolamin and Glutelin.) concentrations (mg/g) measured from *Syzygium cumini L., Lawsonia inermis L. Calotrpis procera.R Bro Ricinus communisL., Linum*

usitatissimum L., Brassica juncea L Czern., Withania coagulans Dun, and Cucumis melo var. agrestis Naudin. seeds found in Sindh, Pakistan.

Conclusion

The findings of the present study reveal that the composition of seed storage proteins differs markedly among the selected medicinal plant of Sindh, Pakistan. Albumin was found to be the dominant protein fraction in *B. juncea L Czern*, suggesting its key role as a primary water-soluble storage protein in this species. Contrastingly, *R. communis L* is highest amount of globulin, suggesting an abundance of salt-soluble proteins. *W. coagulans Dun.* had a unique protein profile, with prolamin being the dominant fraction, suggesting an adaptive mechanism of protein storage. *S.cumini L* had relatively higher levels of glutelin compared to the other species, but glutelin was always the least abundant fraction. This indicates that seed storage protein profiles are species-specific and influenced by genetic and physiological factors, and albumin, globulin, prolamin, and glutelin fractions vary in relation to seed structure, nutritional potential, and metabolic functions.

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