

## ISOLATION AND CHARACTERIZATION OF MAJOR SEED STORAGE PROTEINS: II. APIACEAE FAMILY FOUND IN SINDH, PAKISTAN

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**ABSTRACT:** Seeds of 6 medicinally important plants they belong to family *Apiaceae* growing in Sindh province of Pakistan, including *Anethum graveolens* - dill, *Apium graveolens* - celery, *Coriandrum sativum* - coriander, *Cuminum cyminum* - cumin, *Foeniculum vulgare* -fennel, *Trachyspermum ammi L* - Carom, the deduction of four main seed storage proteins i.e. albumin, globulin, prolamin and glutelin. All plant species found and cultivated in Sindh, Pakistan. Proteins were seed flour was extracted by sequential steps of extractions including delipidation (removal of oil), water (albumin), 5.0 M NaCl (globulin), 70% ethonal (prolamin), and 0.2 M Na<sub>3</sub>PO<sub>4</sub> buffer, pH8.0 (glutelin). Quantitative estimation was performed Dye binding technique of Bradford used for quantifiable estimation and found huge differences in terms of their concentrations and overall production (Table-1). Among all seed plants the albumin fraction was observed high in family *Apiaceae* where, *C. cyminum* (85.01%) and *T. ammi L* (47.12%) containing the highest, *A. graveolans*, *F. vulgare*, *C. sativam L* (37.66%, 36.88%, 30.14%, respectively) contains the medium while, the lowest concentration was observed in *A. graveolans L*. (29.11%). Globulin with the second dominant protein fraction may also vary from 3.52% in *C. cyminum* to 50% in *C. sativam L*. The meaningful increase in prolamin was observed in *T. ammi L* (33.08%), *A. graveolans L* (28.11%), *A. graveolans*, *F. vulgare* (21.34%) while, the lowest of around (9.6%) in *C. cyminum*, *C. sativam L* seeds. On the other hand, a consistent pattern of 5 to 20% of glutelin concentration was detected among every plant seeds (with exception of *A. graveolans L* having 23%). To the help of our research information, this study for the first time reported the comparative seed storage proteins profile of the family *Apiacea* and its possible medicinal and biotechnological application.

**Key words:** *Apiaceae*, Extraction, Protein estimation, Seed storage proteins.

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### INTRODUCTION

*Apiaceae* or Umbelliferae Family (equally titles are acceptable by the ICBN) in this family majority of fragrant plants and with the hollow stems, and this is commonly recognized as umbellifers. Its former name "Umbelliferae" originates specific shape of the inflorescence being mostly in the form of a compound "umbel", and has the resemble root as the word "umbrella". This count a huge family around 300-455 genera and about 3,000-3750 species (Downie *et al.*, 2000). This family consist various highly toxic plants, for example hemlock. wild carrot, have estrogenic things and have been utilized as traditional remedy used for control of the population. Furthermost remarkable for this use is the extinct vast fennel, silphium - prehistoric miracle drug. Plants grown in this class can be measured practically good companion plants, since the tiny flower umbrella attracts useful omnivorous insects. , mostly ladybugs parasitic wasps and predatory flies, which then hunt insect pests near crops (Oroojalian *et al.*, 2010;

Ekiert, 2000).Plants proteins provide approximately 65% of the world's human protein supply with 45 – 50% and 10 – 15% from cereals ,legumes and vegetables, (Sammour, 1999; Casey, 1999; Shewry *et al.*, 1995; Mahe *et al.*, 1994). Plant seeds are the very important source of nutritional proteins and this is essential for complementing protein. Medicinal plants, main source of all related chemical substances which are used in the usage of different harmful diseases. Numerous synthetic medicines that have serious side effects have been described. (Javed *et al.*, 2006). like other ailments, attention is also being bound for to the alternative medicines of herbal origin to discover harmless and inexpensive medications for example hypolipidaemic activity (Javed *et al.*, 2006; Visavadiya & Narasimhacharya, 2005; Rahman and Ghani, 1995; Aftab *et al.*, 1995; Dhandapani *et al.*, 2002). All plant those are related with this family *Apiaceae* are not only a recognized source of numerous important herbal products but also the scientific basis of different biological activities for example anti-microbial, anti-fungal, , anti-inflammatory,

anti-oxidant anti- immunomodulatory and convulsant etc are well recognized (Hemati *et al.*, 2010; Oroojalian *et al.*, 2010; Uma *et al.*, 2009; Dusko *et al.*, 2006; Christensen & Brandt, 2006; Ekiert, 2000; Syed *et al.*, 1986). In the current studies, seeds of six medically important plants that belong to the family *Apiaceae* cultivating in Sindh, Pakistan.

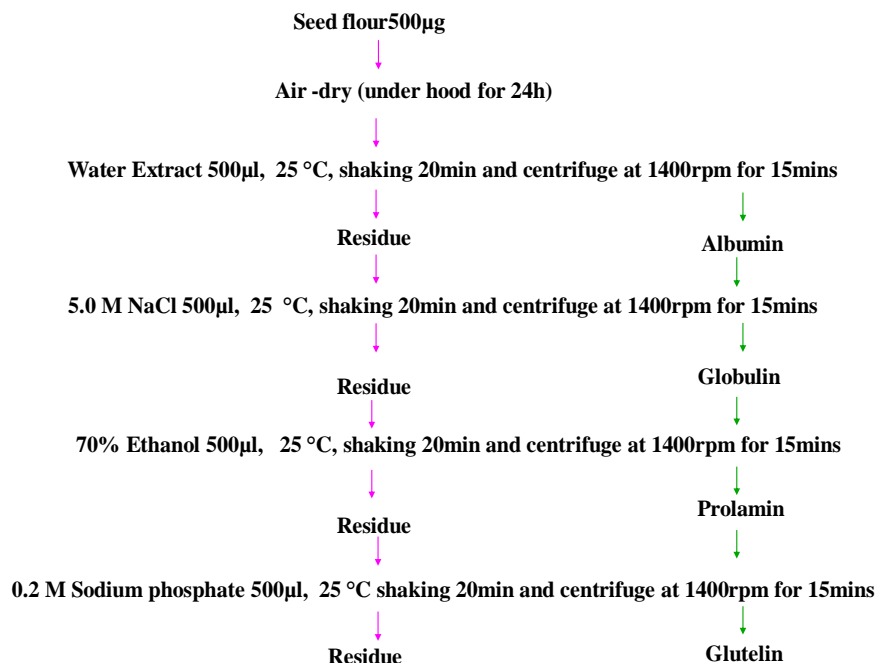
## MATERIAL AND METHODS

**Material:** Seeds of six major plants of family *Apiaceae* namely, *Anethum graveolens*, *Apium graveolens*, *Coriandrum sativum*, *Cuminum cyminum*, *Foeniculum vulgare*, *Trachyspermum ammi L.* were collected. All of these studied plants are agricultural crops of Sindh, and seeds were collected from three different cities Nawabshah, Hyderabad and Karachi. Also a little bit quantity of seeds received by Hamdard University, Karachi.

**Seed storage protein extraction:** The seed was crushed (powder) manually with liquid mortar in liquid nitrogen. The flour was delipidated by solvent extraction by stirring with pure hexane (1:10 w/v), and this procedure time is thirty minutes and repeated for three times at

room temperature. After air drying of the sample, kept under hood upto twenty four hours by using micro-extraction method (Sammour *et al.*, 1999) in alcohol, water, high salt, and in alkaline buffer conditions. Ultimately in triplicate the sample 500 micrograms was extracted in eppendorf tubes with 500 microgram (i) deionized water (ii) 145.0M NaCl (iii) 70% ethanol and (iv) 0.2 M, Na<sub>3</sub>PO<sub>4</sub> buffer, pH8.0 for the extraction of 04 important seed storage proteins such as. Prolamin, albumin, glutelin, and globulin. All extractions were done upto twenty minutes at 350 rpm/25°C using Thermomixer comfort (Eppendorf, Germany). The centrifuge was used for these extraction at 14000 rpm upto 15 min at 4°C (Biofuge Primo R Heraerus, Japan) and after supernatant separated, extraction were repeated twice in order to eliminate residual protein of every fractions. The supernatants of each fraction were pooled and store at -20°C until unless subjected for protein profiling. The whole protein collection from entire extraction was quantified by the altered color binding assay (Bradford, 1976) by bovine serum albumin as a standard. Measurement in triplicate was done by using microplate reader (Sunrise Tecan, Austria).

### EXTRACTION METHOD FOR MAJOR SEED STORAGE PROTEIN (Isolation & Isolation of Seed Storage Proteins) Albumin, Globulin, Prolamin, glutelin



This method is commonly used for improve protein extraction. Seed flour was successively extracted with different buffer conditions; by using modified dye-binding assay of Bradford, the concentration of protein in all extracts were measured.

## RESULTS AND DISCUSSION

In the present study, six medicinally important plants viz. *Apium graveolens*, *Anethum graveolens*,

*Cuminum cyminum*, *Coriandrum sativum*, *Trachyspermum ammi* *Foeniculum vulgare* L related with the family *Apiaceae* were chosen for their main seed storage protein profiling. This technique could better be also used for inter species specific diversity and phylogenetic or evolutionary relationships among various species or genera. Plant proteins must have the required effective properties and offer the necessary amino acids for their use in different systems of nutrition. (Wang and Kinsella, 1976). In developing countries, society relies heavily on unconventional protein sources to increase the convenience of using proteins in their food. Seeds are a very important source of a large amount of protein that can be extracted from seed meal and used as functional ingredients in various nutrients. (Wani *et al.*, 2011). Plant juicing is one of the most important factors driving plant growth. Nitrogen in the plant is best known for its formation in the structure of a protein molecule, so nitrogen plays an essential role in the synthesis of plant components through the action of various enzymes. (Jones *et al.*, 1991). *Anethum graveolens* is a herbal remedy that is especially useful for digestive disorders such as dyspepsia. It is an annual / biennial grass with fur surface and scattered leaves. Its flowers are yellow. This herb is also called Fennel and contains up to 5% volatile oils in its seeds. Its roots contain a-b-pinene essential oils. This herbal medicine is especially used for dyspepsia its fresh and dried leaves are used for gastric, urinary and intestinal problems its aqueous extract drops blood pressure and opens blood vessels. It reduces the heart attack and stimulates breathing. Seed oil is found to have very effective breathing problems. It also works with other respiratory problems, such as colds and bronchitis. Oil of *C. cyminum* and *A. graveolens* is similar or further effective when combined with standard antibiotics at very low concentrations (Singh *et al.*, 2002; Choochote *et al.*, 2004). *Apium graveolens* L. seeds are utilized in Ayurvedic usage of liver diseases (Singh and Handa, 1995). leaf and seeds *A. graveolens* are utilized as a very famous aromatic herb and spice (Rafikali and Muraleedharan, 2001; Kitajima *et al.*, 2003). Bioactive compounds consequent from *A. graveolens* seeds have also been recognized to possess nematocidal activity against *Panagrellus redivivus* and *Caenorhabditis elegans*, antifungal activity against *Candida albicans* (Rafikali *et al.*, 2000; Rafikali and Muraleedharan, 2001; Kitajima *et al.*, 2003). The Coriander (*Coriandrum sativum*) The plant grows throughout for seed seed, as an essential oil, as well as a spice (Bhuiyan *et al.*, 2009). The main ingredients obtained from fruit include linalool and some other oxygen monoterpenes and monoterpene hydrocarbons (Bandoni *et al.*, 1998; Anitescu *et al.*, 1997). Almost people are using it as a medicine. Otherside it is also found effective against bacteria and used as an ingredients in different herbal products such as lotions and shampoo (herbal) (Chopra *et*

*al.*, 1956). *Funicular vulgare* (fennel) very greatest important medicinal plants (Omidbaigi, 2005). The plant has rich applications in various industries. For example, essential oil from seeds is added to perfumes, pharmaceuticals, cosmetics and soaps, fennel extracts oil; The seeds are also mostly used for flavored foods, including meat and ice cream, sweets, baked goods, and are also used as a flavor for various spices.

This work has shown that the essential oil of this plant can be used as a valuable, antibacterial antioxidant and anti-body. This work has shown that the essential oil of this plant can be utilized as a valuable, antibacterial antioxidant and for the antifungal (Lucinewton *et al.*, 2005). The important oil of the plant is anethole and fenchone (Lewinshon *et al.*, 2001; Simandi *et al.*, 1999). Fennel extracts evidenced to have anti-inflammatory, antispasmodic, carminative, diuretic, expectorant, laxative, analgesic, stimulant of gastrointestinal mobility and almost utilized for the nervous disorders (Choi and Hwang, 2004). Anti-cancer activity of fennel seed antennal was also recently reported (Anand *et al.*, 2008).

*The Cuminum cyminum* (Cumin) is commonly used in Ayurveda remedy for the treatment of dyspepsia, jaundice and diarrhoea (Dhandapani *et al.*, 2002). It is used in many veterinary medicines as carminative, stomach, athletic, and it is very useful against diarrhea and constipation. Cumin Seed has a strong aromatic spice aroma, and a long history of use as a medicinal herb. In traditional herbal medicine, Cumin seeds are also studied for their anti-cancer properties. It reduces superficial inflammation and pain. Kaman seeds work on the female reproductive system, decreasing swelling of the uterus. It's commendable due to the lactagogue qualities, and cause of milk increase among lactating mothers. *Trachyspermum ammi* L (Sprague), known as Ajwain, is known to have an inhibitory effect on platelet aggregation. (Srivastava, 1988), antifungal potency (Dwivedi and Dubey, 1993) and blood pressure lowering action (Rahman and Ghani, 1995). In India the essential oil and its main component (thymol) used as medicine, particularly for cholera (Lawless, 1992). *T. ammi* L. seeds are small in size. It tastes bitter, hot, pouring, mild, raw, gastric, anthelmintic and aphrodisiac. Seeds can treat certain diseases of the mouth and heart, vomiting, ascites, piles, abdominal cancer and pain. It should be taken in small doses, as in some people, it can cause irritation of the skin. The seed storage proteins are commonly categorized in four groups according to their water salt soluble, solubility soluble, globulin, plus albumin, prolamin alcohol soluble and glutelin alkaline soluble proteins. Seed flour was extracted by consecutive phases of extractions with delipidation (removal of oil), H<sub>2</sub>O (albumin), 5.0 M NaCl (globulin), 70% ethanol (prolamin), and 0.2 M (Na<sub>3</sub>PO<sub>4</sub>) buffer, pH8.0 (glutelin). Dye binding technique of Bradford used for quantifiable estimation and found huge differences in terms of their

concentrations and overall production (Table-1). Among all seed plants the albumin fraction was observed high in family Apiaceae where, *C. cyminum* (85.01%) and *T. ammi* L (47.12%) containing the highest, *A. graveolans*, *F. vulgare*, *C. sativum* L (37.66%, 36.88%, 30.14%, respectively) contains the medium while, the lowest concentration was observed in *A. graveolans* L. (29.11%). Globulin with the second dominant protein fraction may also vary from 3.52% in *C. cyminum* to 50% in *C. sativum* L. Similarly, the prolamin result was observed very greater in *T. ammi* L. (33.08%), *A. graveolans* L (28.11%), *A. graveolans*, *F. vulgare* (21.34%) while, the lowest of around (9.6%) in *C. cyminum*, *C. sativum* L seeds. On the other hand, a

consistent pattern of 5-20% of glutelin concentration was detected in all seeds of plants (with exception of *A. graveolans* L having 23%). Prolamin which is well-known by its alcohol solubility from the three other different classes of seed storage proteins i.e. the glutelins dilute alkali globulins (salt-soluble), and albumins (water-soluble), (Caasey, 1999; Shewry *et al.*, 1995). The albumin and globulin are richly present in angiospermic plants both in monocot and dicot plant seeds including cereals, palms, fern spores (Templeman *et al.*, 1987), provide all basic needs of life such as food, shelter, fodder, timber, medicines and fuel etc (Iqbal and Hamayon, 2006).

**Table 1. Comparison of the concentration and total percent yield of the major seed storage proteins in the family Apiaceae found in Sindh, Pakistan.**

S. No	Plants Name	Common Name	Albumin		Globulin		Prolamin		Glutelin	
			mg/g*	% yield	mg/g	% yield	mg/g	% yield	mg/g	% yield <sup>#</sup>
1	<i>Anethum graveolens</i> L.	Dill/Sowa	0.55	37.66	0.38	26.00	0.31	21.34	0.21	14.97
2	<i>Apium graveolans</i> L.	Celery/Ajmud	0.27	29.11	0.18	19.28	0.26	28.11	0.22	2348
3	<i>Cuminum cyminum</i>	Cumin/Zeera	0.27	85.01	0.01	3.52	0.03	9.60	0.00	1.86
4	<i>Foeniculum vulgare</i> Mill.	Fennel/ Saunf	0.35	36.88	0.20	21.46	0.20	21.41	0.19	20.24
5	<i>Corianderum sativum</i> L.	Coriander/Dhnaea	0.72	30.14	1.20	50.16	0.23	9.64	1.0	10.04
6	<i>Trachyspermum ammi</i> L	Carom/Ajowan	0.18	47.12	0.05	14.68	0.13	33.08	0.02	5.10

\*Concentrations in mg/g of seed flour. <sup>#</sup> Percent (%) yield of a particular protein in total protein contents of seed flour. Values are mean of three independent extractions.

**Conclusion:** As per results, all of these six plants keep good source of seed storage proteins in family *Apiaceae* and provide information of these medicinal important plants could be used as an alternative sources for human diet after proper processing the different species of seeds which contain different concentrations in different physiological conditions. It is worth mentioning here that these seeds are also used as a medicine and home remedies for the gastrointestinal problems; such as colic pain, indigestion and flatulence. It can be used as a flavor, aroma as well as preservative in food commodities. Also known as an antimicrobial activity has reported for this plant/essential oil. However, the extraction from seeds is using as a traditional home remedy without any scientific rational. Thus, to provide a scientific justification for these traditional remedies, estimation of seed storage proteins were performed to provide a basis for improving the dietary and processing properties of crops by various available genetic engineering apparatuses.

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