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 cultured 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% ethanol (prolamin), and 0.2 M Na₃PO₄ buffer, pH 8.0 (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 Apiaceae 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. Furthermore most 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; Mahé 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 & Narasimhabitarity, 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- immunemodulatory 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 up to 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, Na₂PO₄ buffer, pH8.0 for the extraction of 04 important seed storage proteins such as. Prolamin, albumin, glutelin, and globulin. All extractions were done up to twenty minutes at 350 rpm/25°C using Thermomixer comfort (Eppendorf, Germany). The centrifuge was used for these extraction at 14000 rpm up to 15 min at 4°C (Biofuge Primo R Heraeus, 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).

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**EXTRACTION METHOD FOR MAJOR SEED STORAGE PROTEIN**

<table>
<thead>
<tr>
<th><strong>Extraction &amp; Isolation of Seed Storage Proteins</strong></th>
<th><strong>Albumin</strong>, <strong>Glublin</strong>, <strong>Prolamin</strong>, <strong>Glutelin</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed flour 500µg</td>
<td></td>
</tr>
<tr>
<td>Air-dry (under hood for 24h)</td>
<td></td>
</tr>
<tr>
<td>Water Extract 500µl, 25°C, shaking 20min and centrifuge at 1400rpm for 15mins</td>
<td>Albumin</td>
</tr>
<tr>
<td>Residue</td>
<td></td>
</tr>
<tr>
<td>5.0 M NaCl 500µl, 25°C, shaking 20min and centrifuge at 1400rpm for 15mins</td>
<td>Globulin</td>
</tr>
<tr>
<td>Residue</td>
<td></td>
</tr>
<tr>
<td>70% Ethanol 500µl, 25°C, shaking 20min and centrifuge at 1400rpm for 15mins</td>
<td>Prolamin</td>
</tr>
<tr>
<td>Residue</td>
<td></td>
</tr>
<tr>
<td>0.2 M Sodium phosphate 500µl, 25°C shaking 20min and centrifuge at 1400rpm for 15mins</td>
<td>Glutelin</td>
</tr>
<tr>
<td>Residue</td>
<td></td>
</tr>
</tbody>
</table>

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 choose 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-pine 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 Muraleednaran, 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 albican (Rafikali et al., 2000; Rafikali and Muraleednaran, 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 (Bhuiany et al., 2009). The main ingredients obtained from fruity fruits 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), H2O (albumin), 5.0 M NaCl (globulin), 70% ethanol (prolamin), and 0.2 M (Na3PO4) 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. cuminum (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. cuminum to 50% in C. sativam 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. cuminum, C. sativam 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.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Plants Name</th>
<th>Common Name</th>
<th>Albumin mg/g %yield</th>
<th>Globulin mg/g %yield</th>
<th>Prolamin mg/g %yield</th>
<th>Glutelin mg/g %yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anethum graveolens L.</td>
<td>Dill/Sowa</td>
<td>0.55 37.66</td>
<td>0.38 26.00</td>
<td>0.31 21.34</td>
<td>0.21 14.97</td>
</tr>
<tr>
<td>2</td>
<td>Apium graveolans L.</td>
<td>Celery/Ajmuud</td>
<td>0.27 29.11</td>
<td>0.18 19.28</td>
<td>0.26 28.11</td>
<td>0.22 23.48</td>
</tr>
<tr>
<td>3</td>
<td>Cuminum cuminum</td>
<td>Cumin/Zeera</td>
<td>0.27 85.01</td>
<td>0.01 3.52</td>
<td>0.03 9.60</td>
<td>0.00 1.86</td>
</tr>
<tr>
<td>4</td>
<td>Foeniculum vulgare Mill.</td>
<td>Fennel/Saunf</td>
<td>0.35 36.88</td>
<td>0.20 21.46</td>
<td>0.20 21.41</td>
<td>0.19 20.24</td>
</tr>
<tr>
<td>5</td>
<td>Corianderum sativum L.</td>
<td>Coriander/Dhnnea</td>
<td>0.72 30.14</td>
<td>1.20 50.16</td>
<td>0.23 9.64</td>
<td>1.0 10.04</td>
</tr>
<tr>
<td>6</td>
<td>Trachyspermum ammi L.</td>
<td>Carom/Ajowan</td>
<td>0.18 47.12</td>
<td>0.05 14.68</td>
<td>0.13 33.08</td>
<td>0.02 5.10</td>
</tr>
</tbody>
</table>

*Concentrations in mg/g of seed flour. * 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 preservator 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.

**REFERENCES**


