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Partial Amino Acid Sequence of Lipid Transfer Protein from Fennel (*Foeniculum vulgare*) Seeds

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ABSTRACT

Fennel (*Foeniculum vulgare*) is a biennial Egyptian medicinal plant with an aromatic odor that belongs to the family Apiaceae (Umbelliferae). Fennel seeds are commonly used in traditional medicine, as they are known to have anti-inflammatory, anti-fungal and anti-cancerous activities. The major constituents of the fennel plant are sugars, minerals, essential fatty acids, proteins and fibers. Although, there are numerous studies on the medicinal properties of essential oils of the fennel seeds, but there is limited data reported on the proteins and peptides. The aims of this project are to fully characterize the primary structure of proteins and to determine their biological activities. We present here the preliminary data on the amino acid sequence of Lipid Transfer Protein (LTP) from fennel seeds. The proteins were extracted in Tris/HCl pH 8 buffer and successfully purified by two-dimensional liquid chromatography (2D-LC), using gel filtration chromatography followed by reversed phase HPLC (RP-HPLC). The purity of isolated LTP protein was determined by the SDS gel electrophoresis. The purified protein was loaded on to the PVDF disc and sequenced by automated amino acid sequencer, model PPSQ-31A (Shimadzu). The partial amino acid sequence up to 22 amino acid residues was successfully established. The amino acid sequence similarity was searched using Protein BLAST, which revealed it to be a lipid transfer protein.

METHOD

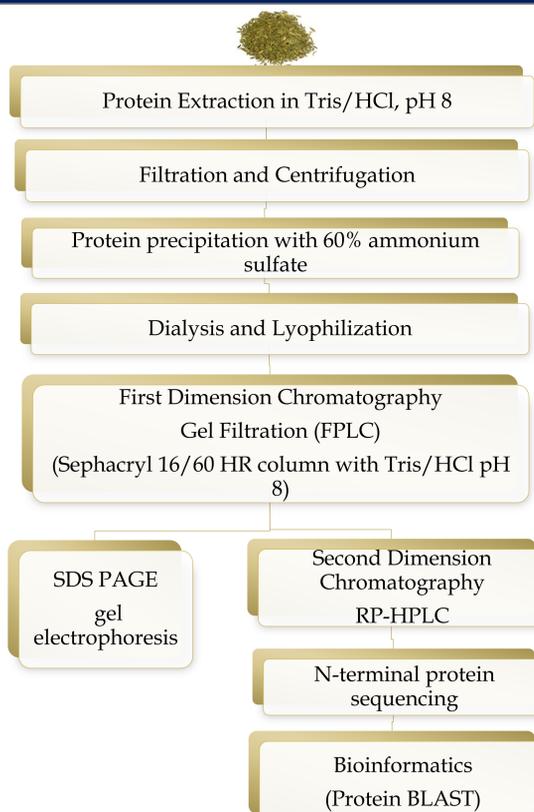


Figure 1: Schematic of Workflow

RESULTS

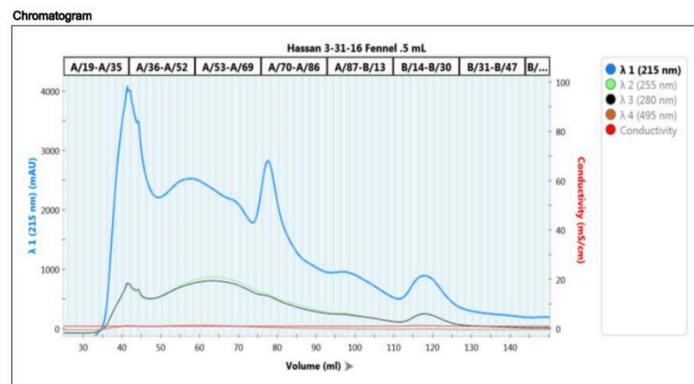


Figure 2: Protein elution profile of fennel extract by gel filtration chromatography. Column HiPrep 16/60 Sephacryl S-200HR; isocratic elution buffer 20mM Tris/HCl pH 8.0; flow rate 0.5 ml/min.

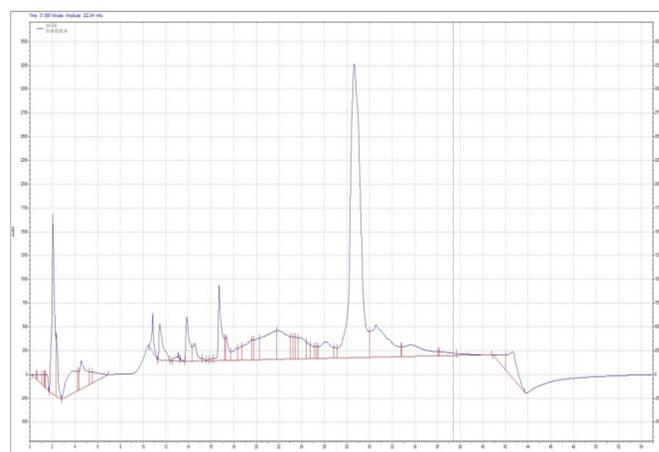


Figure 4: Protein elution profile of gel filtration fractions 75-80 by RP-HPLC. Column Aeries Protein-C18 (4.6x250 mm). Solvent A: 0.1% TFA-water, Solvent B 0.1% TFA-acetonitrile. Gradient elution of 0-60% B in 60 min; flow rate 1 ml/min; absorbance monitored at 214 nm.

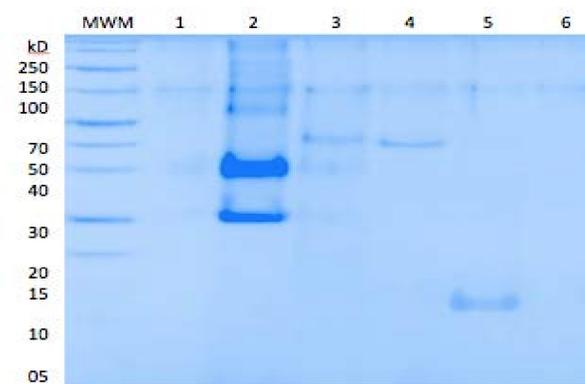


Figure 3: SDS PAGE of Fennel fractions after gel filtration chromatography (FPLC). Lane 1: Molecular Weight Marker (5-250 kDa). Lane 2: 20 µl A17, Lane 3: 20 µl A40, Lane 4: 20 µl A60, Lane 5: 20 µl A75, Lane 6: 20 µl A80.

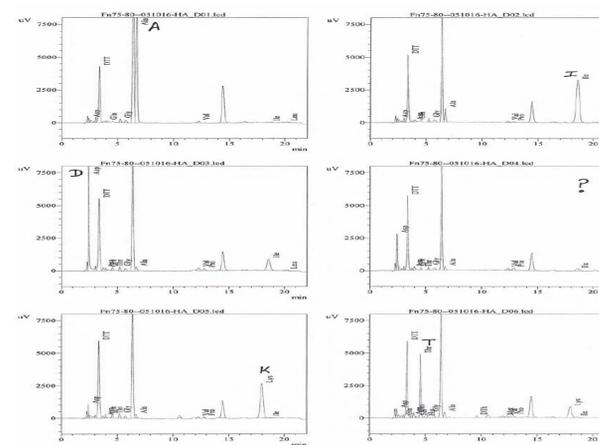


Figure 5: PTH amino acid elution profile of cycles 1-6 from automated protein sequencer PPSQ-31A (Shimadzu).

Download	GenPept	Graphics
PREDICTED: non-specific lipid-transfer protein A-like [Nicotiana tomentosiformis] Sequence ID: reflXP_009623063.1 Length: 112 Number of Matches: 1		
Range 1: 21 to 42	GenPept	Graphics
Score	Expect	Identities
49.8 bits(110)	1e-05	17/23(77%)
		Positives
		18/22(81%)
		Gaps
		0/22(0%)
Query 1	AIDCKTVDAALVPCVPLTGGG	22
	AI+C VDA LVPCVPLT GG	
Sbjct 21	AINCGQVDTLVPCVPLTGGG	42

Download	GenPept	Graphics
hypothetical protein DCAR_029747 [Daucus carota subsp. sativus] Sequence ID: gb KZM82178.1 Length: 110 Number of Matches: 1		
Range 1: 21 to 43	GenPept	Graphics
Score	Expect	Identities
49.0 bits(108)	3e-05	17/23(74%)
		Positives
		17/23(73%)
		Gaps
		0/23(0%)
Query 1	AIDCKTVDAALVPCVPLTGGG	23
	A DC VD LVPCVPLT GGT	
Sbjct 21	ALDCGKVDLFLVPCVPLTAGGT	43

Figure 6: Amino acid sequence similarity search of 22 amino acid residues using Protein BLAST.

DISCUSSION

Our research group is interested in the characterization of biologically active proteins and peptides from various plant sources including leaves, roots, and seeds. In this poster, the partial amino acid sequence of a purified non-specific lipid transfer protein (n-LTP) from fennel seeds is presented. The schematic of workflow used to purify and characterize the Fennel seeds (n-LTP) protein is shown in Figure 1.

As an initial step for protein purification, FPLC based gel filtration chromatography was employed. This technique was chosen because it separates proteins on the basis of protein molecular weight, which removes small molecular weight impurities, including color components. The elution profile by gel filtration chromatography using HiPrep 16/60 Sephacryl S-200 is shown in Figure 2. There are five distinct peaks, indicating that there are at least five different proteins present. Although each peak is very distinct, each peak could contain multiple proteins. The purity of isolated peaks was confirmed by SDS PAGE gel electrophoresis. The electrophoretic profile of gel filtration fraction 75, shows a single band (Figure 3).

The gel filtration fractions (75-80) was pooled and further purified by second dimensional liquid chromatography (2D-LC) using RP-HPLC. The column used was Aeries Protein-C18 (4.6x250 mm) and a linear gradient of acetonitrile from 0-60% in 60 min was employed (Figure 4). The one distinct peak eluted at 29 minutes, indicating the presence of a purified protein. This peak was loaded onto the automatic protein sequencer. The identities of the first 22 amino acids were revealed as shown in Figure 5. The amino acid sequence established was Ala-Leu-Asp-Cys-Lys-Thr-Val-Asp-Ala-Ala-Leu-Val-Pro-Cys-Val-Pro-Tyr-Leu-Thr-Gly-Gly-Gly. Finally, the sequence similarity was searched using Protein BLAST confirmed the identity of this protein as a non-specific lipid transfer protein (n-LTP). It was further revealed that partial sequence of the fennel protein has about an 80% match with the predicted sequences from other plants as shown in Figure 6. Further studies to determine the complete primary structure of the n-LTP as well as biological assay are in progress.

ACKNOWLEDGMENTS

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