

**The structural characterization and bioactivity assessment of nonspecific lipid transfer protein 1 (nsLTP1) from caraway (*Carum carvi*) seeds**

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**Supplementary Table S1:** Template sequences obtained by BLASTp results against PDB database.

<b>S. No.</b>	<b>Organism Name</b>	<b>Total score</b>	<b>Query coverage</b>	<b>E-value</b>	<b>Percent Identity</b>	<b>Positives</b>	<b>PDB ID</b>
1.	<i>Solanum melongena</i>	100	97%	6E-29	47.78	67	5TVI_V
2.	<i>Actinidia chinensis</i> var. <i>chinensis</i>	100	97%	7E-29	50.00	68	7KSB_A
3.	<i>Prunus persica</i>	76.3	97%	3E-19	40.00	58	2ALG_A
4.	<i>Lens culinaris</i>	72.8	97%	6E-18	42.22	55	5LQV_A
5.	<i>Lens culinaris</i>	72.8	97%	6E-18	42.22	55	2MAL_A
6.	<i>Corylus avellana</i>	70.5	98%	5E-17	38.46	54	4XUW_A
7.	<i>Oryza sativa</i>	70.1	97%	6E-17	37.78	56	1BV2_A
8.	<i>Zea mays</i>	70.1	97%	8E-17	39.56	58	1AFH_A
9.	<i>Triticum aestivum</i>	68.9	97%	1E-16	35.56	58	1BWO_A
10.	<i>Artemisia vulgaris</i>	65.9	97%	3E-15	36.67	53	6FRR_A
11.	<i>Vigna radiata</i> var. <i>radiate</i>	65.5	97%	4E-15	38.89	53	1SIY_A
12.	<i>Pisum sativum</i>	63.5	97%	3E-14	39.13	56	2N81_A
13.	<i>Hordeum vulgare</i>	61.2	97%	2E-13	31.11	55	1BE2_A
14.	<i>Punica granatum</i>	59.7	97%	8E-13	34.07	54	7KSC_A
15.	<i>Nicotiana tabacum</i>	58.9	97%	1E-12	36.67	54	1T12_A
16.	<i>Anethum graveolens</i>	57.8	97%	4E-12	36.56	52	2N2Z_A

**Supplementary Table S2:** The molecular interactions found between caraway nsLTP1 and linolenic, linoleic, stearic and palmitic acids ligands.

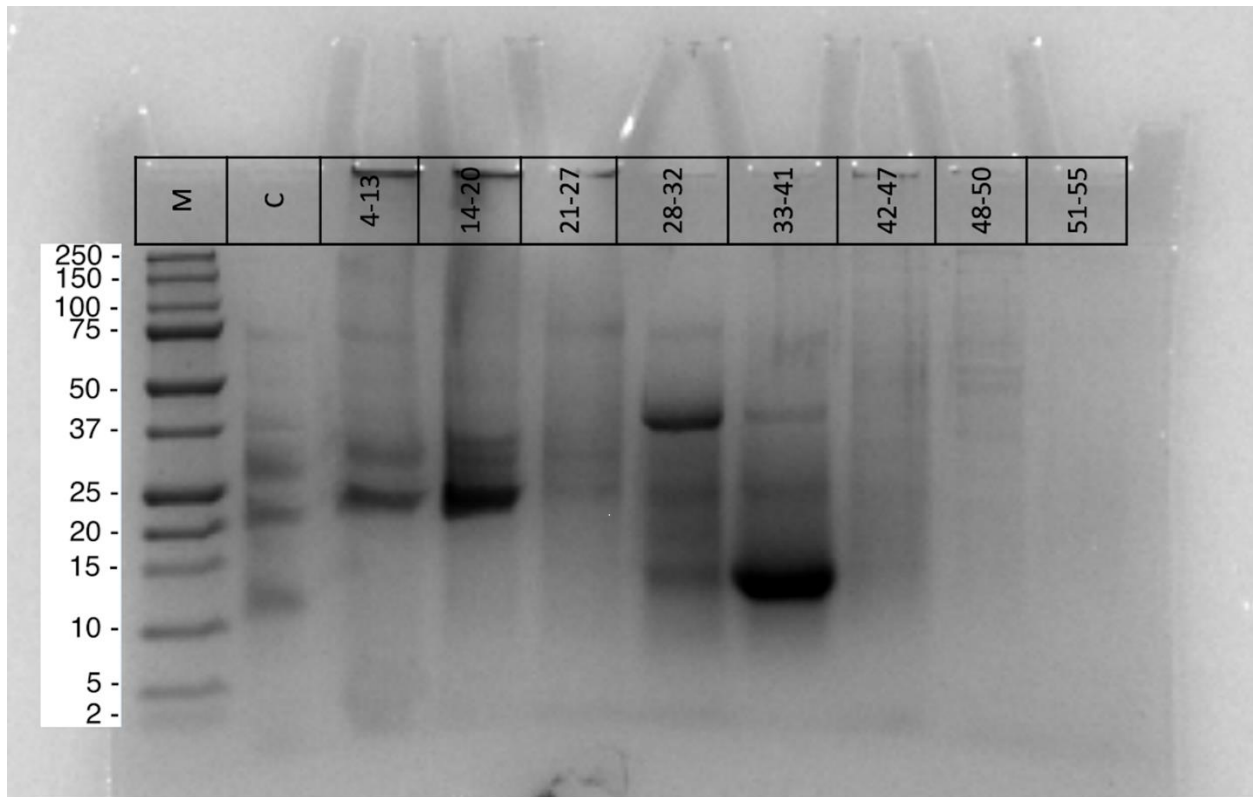
<b>COMPOUNDS</b>	<b>VINA SCORES</b>	<b>HYDROGEN BOND (Å)</b>	<b>HYDROPHOBIC INTERACTIONS</b>	<b>VAN DER WAALS INTERACTIONS</b>
Linolenic acid	-3.6 Kcal/mol	Leu78 (2.1 Å)	Pro71 (Alkyl) Pro81 (Alkyl)	Ala67, Gln68, Lys77, Ile82 and Ser83
Linoleic acid	-3.5 Kcal/mol	Leu78 (2.8 Å)	Pro71 (Alkyl) Pro81 (Alkyl) Ile91 (Alkyl)	Ala67, Gln68, Asp79, Ile82, Ser83 and Thr86
Stearic acid	-3.4 Kcal/mol	Thr86 (1.9 Å)	Ala67 (Alkyl) Pro71 (Alkyl) Pro81 (Alkyl)	Asp64, Gln68, Leu78, Ile82, Ser83 and Asn85
Palmitic acid	-3.0 Kcal/mol	Leu78 (2.0 Å)	Ala67 (Alkyl) Pro71 (Alkyl) Pro81 (Alkyl)	Asp64, Gln68, Lys77, Ile82, Ser83, Asn85 and Thr86

**Supplementary Table S3:** Artificial fluids affect over caraway nsLTP1 secondary structure estimated by Principal Component Regression (PCR) analysis of CD spectra.

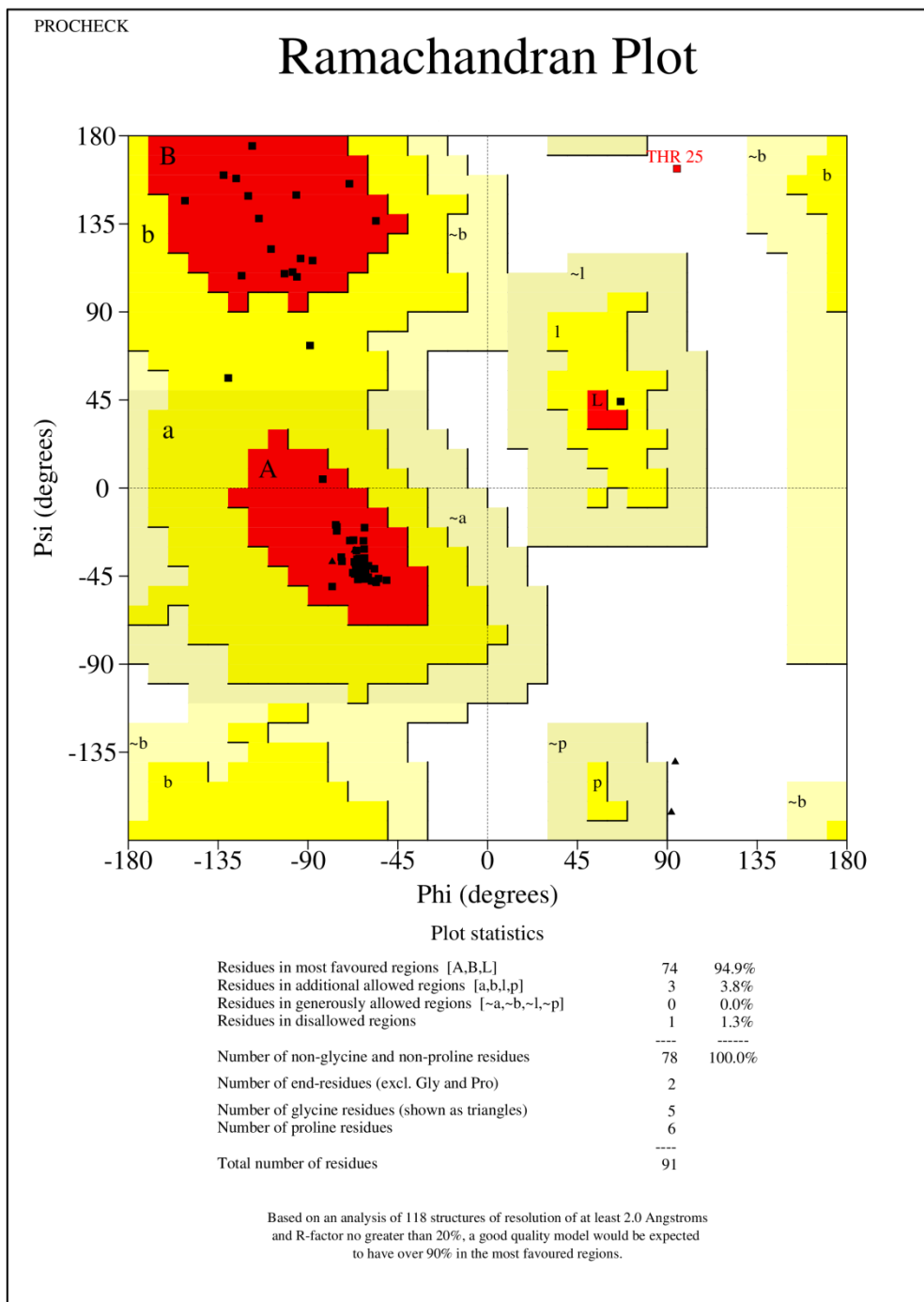
Temperature	$\alpha$ -helix	$\beta$ -sheet	Turn	Other
Ultrapure Water	32.4%	21.9%	11.2%	34.5%
AIF*	46.4%	2.4%	15.3%	35.9%
AGF*	37.3%	18.4%	13.0%	31.3%

\*Artificial Gastric (AGF) and Intestinal (AIF) Fluids.

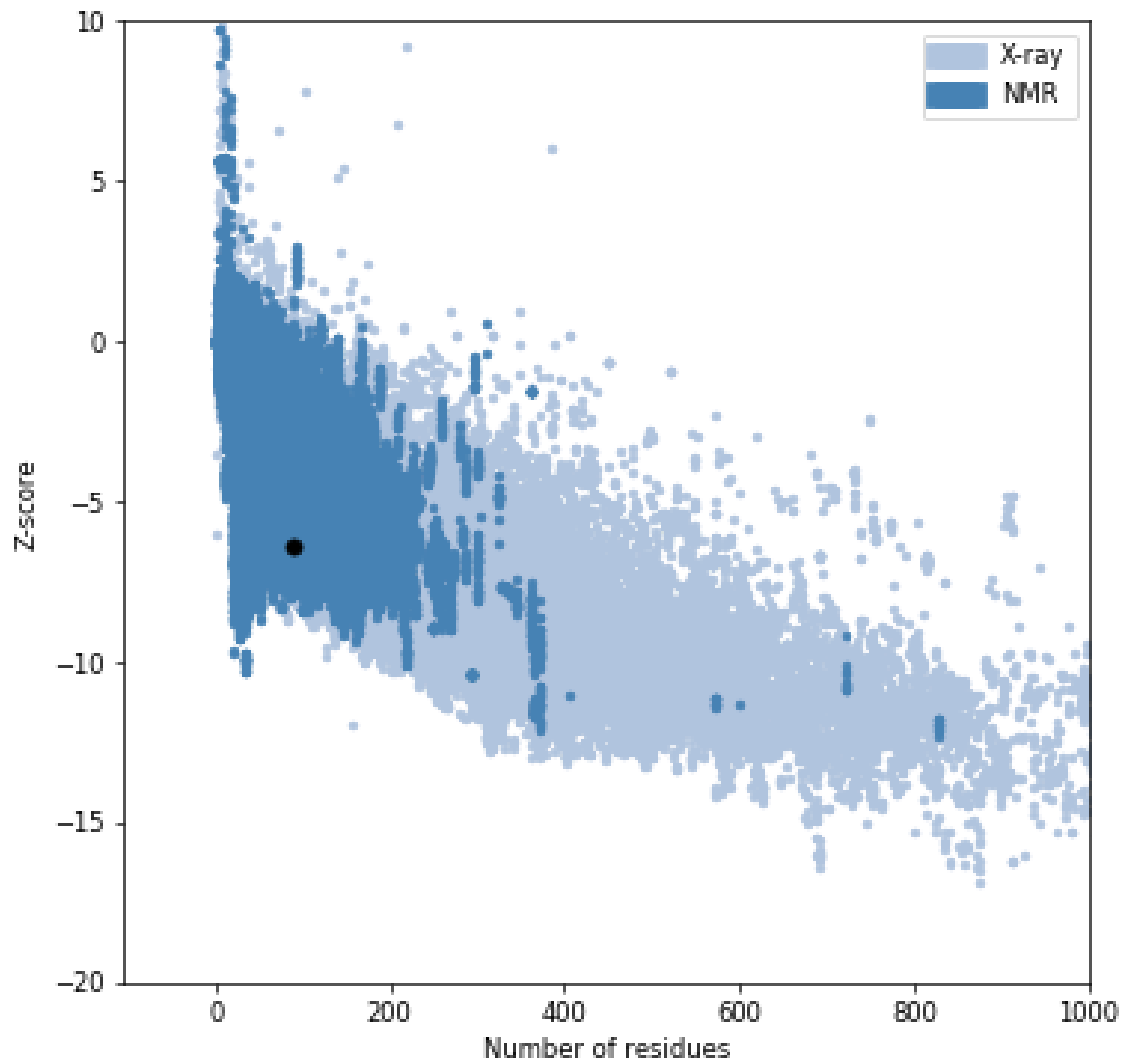
**Supplementary Fig. S1.** The full-length electrophoretic profile by Tris/Tricine SDS-PAGE (10%) of caraway seeds proteins precipitates and gel filtration chromatography fractions. Lane M, standard molecular weight marker, Lane C, crude proteins, and Lane 1–8, eluted gel filtration fractions.



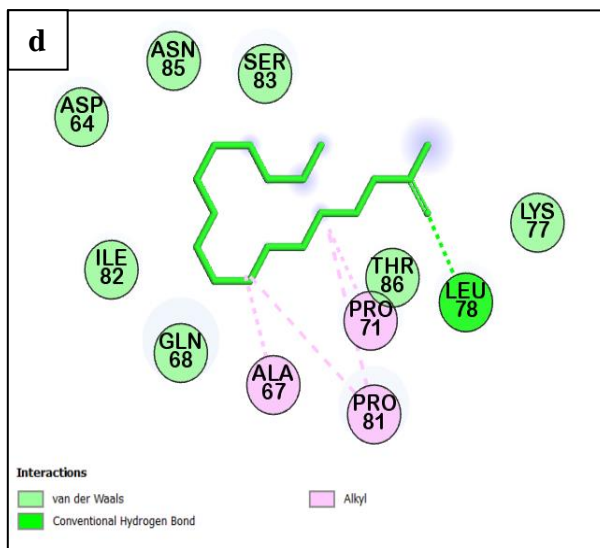
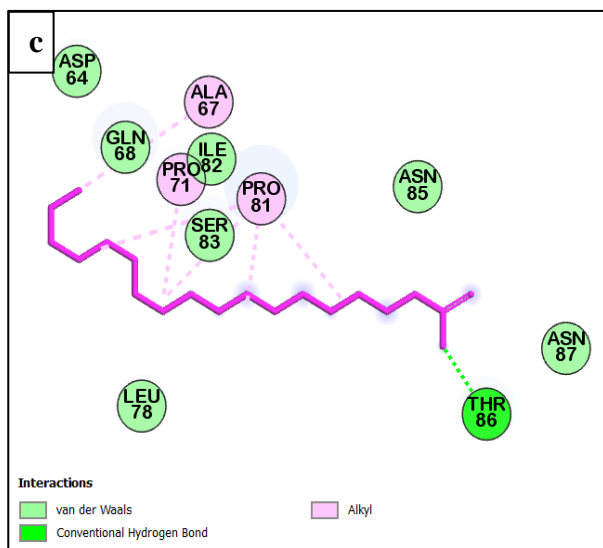
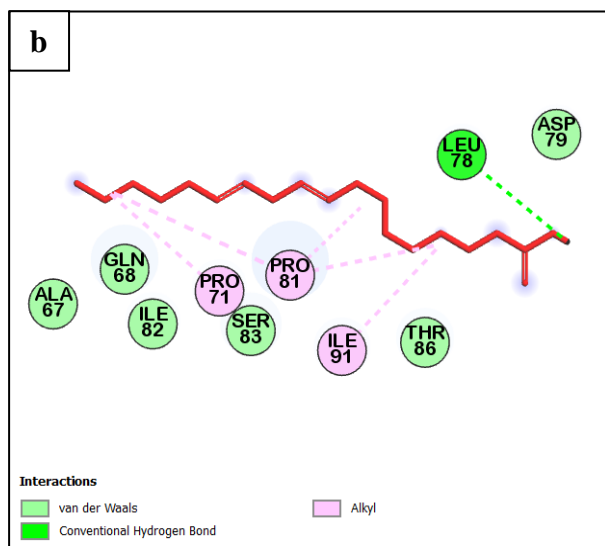
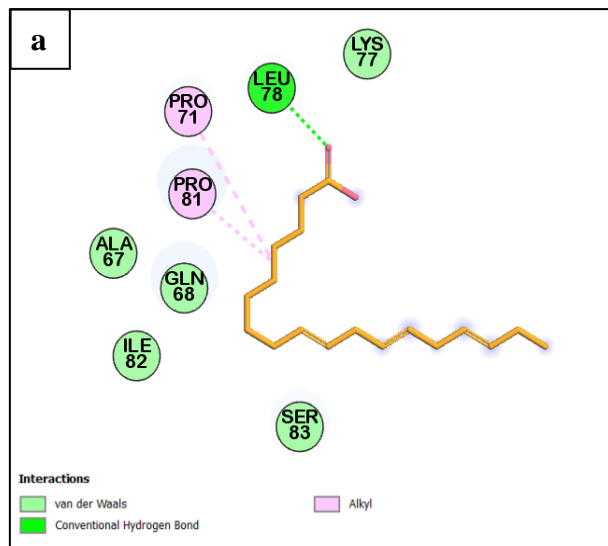
**Supplementary Fig. S2.** Ramachandran Plot of modeled structure of caraway nsLTP1. Most favored region contains more than 90% residues predicting a good quality model.



**Supplementary Fig. S3.** Validation of modeled caraway nsLTP1 structure through ProSA server. Z-score is -6.37 within the range of scores of experimentally determined structures.



**Supplementary Fig. S4.** 2D interactions drawn from Discovery Studio of four fatty acids complexed with caraway nsLTP1 (a) linolenic acid (b) linoleic acid (c) stearic acid (d) palmitic acid are making hydrogen bond, van der waal interaction and alkyl (hydrophobic) interaction.





**Supplementary Fig. S5.** Stability of caraway nsLTP1 under different pH environments. Caraway nsLTP1 in ultrapure water, artificial gastric fluid pH 1.6, and artificial intestinal fluid pH 6.8 CD spectra were collected at 37 °C.

