

Isolation and Identification of a Novel Anti-Dry Eye Peptide from Tilapia Skin Peptides Based on In Silico, In Vitro, and In Vivo Approaches

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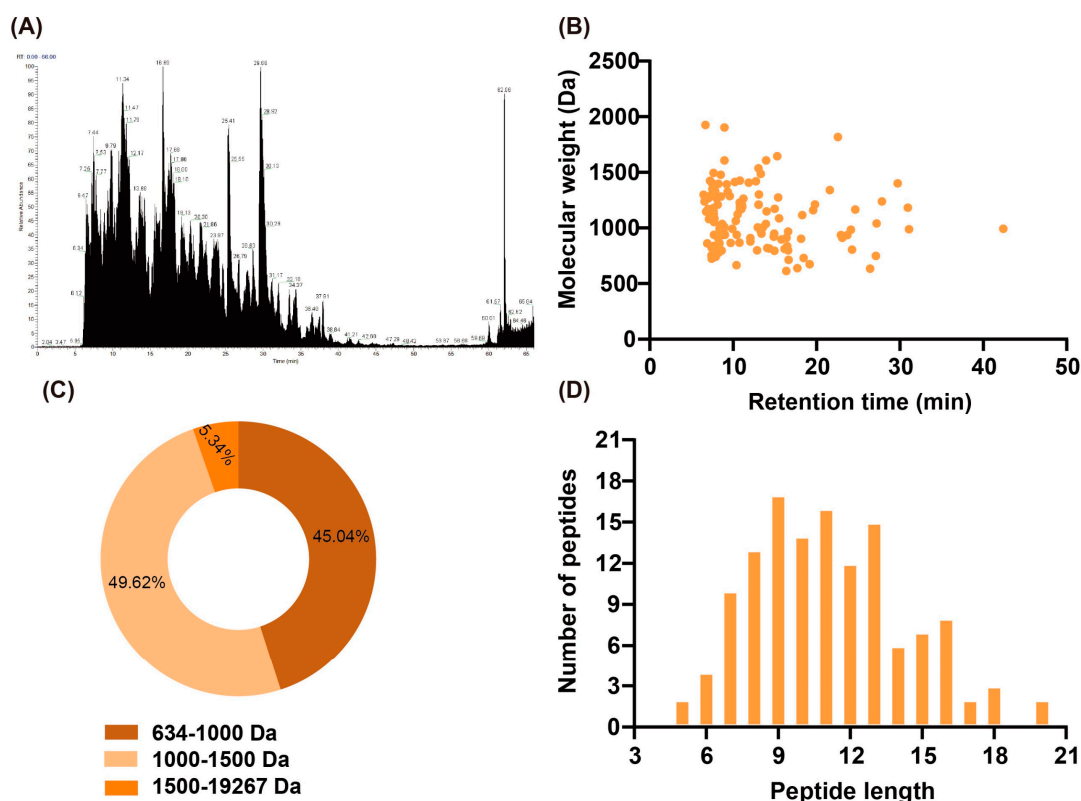


Figure. S1 Result of LC/MS/MS identification of tilapia skin peptides-II (TSP-II). **(A)** Total ion flow diagram of TSP-II; **(B,C)**. Distribution of molecular weight of TSP-II. **(D)** Distribution of the length of amino acids for TSP-II.

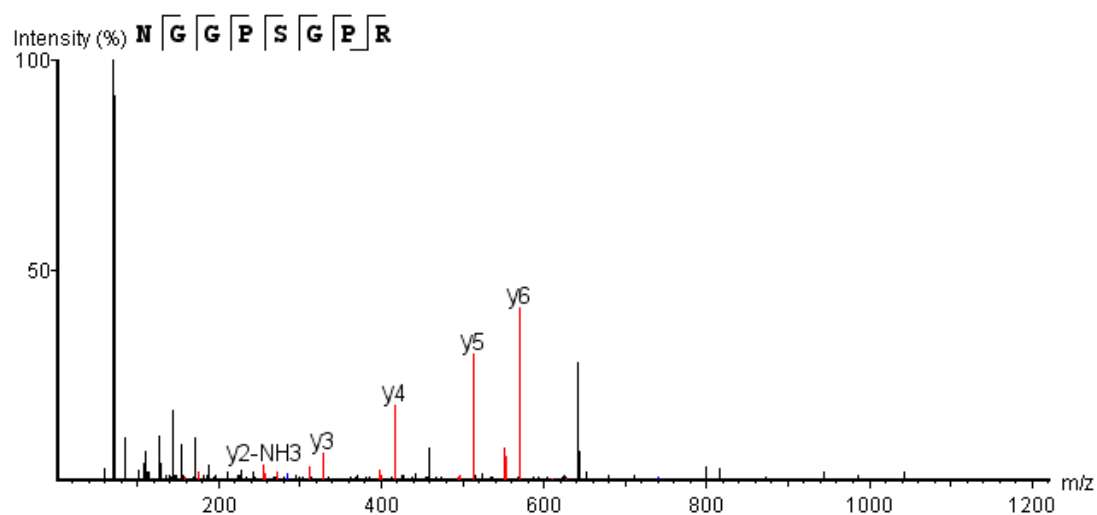


Figure. S2 The result of LC/MS/MS for NGGPSGPR (NGG).

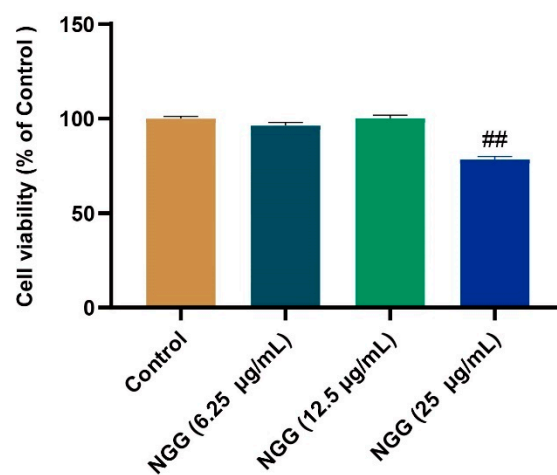


Figure. S3 Results of toxicity of NGG on Human corneal epithelial cells. $n = 5$, ^{##} $p < 0.01$, vs control group.

Table S1. Sequence of TSP-II and its prediction by PeptideRanker and ToxinPred.

| NO. | Peptide Sequence | Mass (Da) | PeptideRanker Score | ToxinPred |
|-----|------------------|-----------|---------------------|-----------|
| 1 | GPAGGPLGPR | 877.4769 | 0.899783 | Non-Toxin |
| 2 | AGPGAGPLGPR | 948.514 | 0.898817 | Non-Toxin |
| 3 | GPAGPAGPGGPPSGR | 1230.6104 | 0.885754 | Non-Toxin |
| 4 | AGPRGPAGPSGLR | 1191.6472 | 0.884835 | Non-Toxin |
| 5 | DFCPPGFNTK | 1181.5176 | 0.88423 | Non-Toxin |
| 6 | QGPAGGPLGPR | 1005.5355 | 0.880138 | Non-Toxin |
| 7 | KAPDPF | 673.3435 | 0.867679 | Non-Toxin |
| 8 | SGPAGPAGPGGPPRQ | 1301.6475 | 0.861916 | Non-Toxin |
| 9 | QSGPAGPAGPGGPPQR | 1486.7275 | 0.841318 | Non-Toxin |
| 10 | SDFPALR | 804.413 | 0.832621 | Non-Toxin |
| 11 | AGPEGPLGPR | 949.4981 | 0.811454 | Non-Toxin |

| | | | | |
|----|----------------------|-----------|----------|-----------|
| 12 | AGPSGPAGRP | 865.4406 | 0.807969 | Non-Toxin |
| 13 | QGGGPQGPVGTGPKG | 1406.6902 | 0.790962 | Non-Toxin |
| 14 | GPAGPAGRPGPSGE | 1205.5789 | 0.784207 | Non-Toxin |
| 15 | VFPGLPDHL | 993.5283 | 0.776531 | Non-Toxin |
| 16 | GPAGPRGPA | 778.4085 | 0.762096 | Non-Toxin |
| 17 | PGPSGPKGEPGAK | 1177.6091 | 0.762084 | Non-Toxin |
| 18 | GPPGTPGSPGPGFEGGKPQ | 1816.8743 | 0.760928 | Non-Toxin |
| 19 | NSAGPQGPLGPR | 1149.5891 | 0.751392 | Non-Toxin |
| 20 | GPAGPRSGPGAA | 993.4991 | 0.742922 | Non-Toxin |
| 21 | GPSGPRGPA | 794.4034 | 0.742249 | Non-Toxin |
| 22 | QHGPSGPRGPAGPHGPVGKD | 1903.9402 | 0.740727 | Non-Toxin |
| 23 | GPRGPAGPHGPVGKD | 1397.7163 | 0.732285 | Non-Toxin |
| 24 | GPSGRPGPAGPH | 1085.5366 | 0.722768 | Non-Toxin |
| 25 | AGPAGPRGSPGA | 993.4991 | 0.71577 | Non-Toxin |
| 26 | AGPRGASGPAGPR | 1149.6003 | 0.70826 | Non-Toxin |
| 27 | LGPAGPR | 666.3813 | 0.705134 | Non-Toxin |
| 28 | AGPAGPRGSPGAA | 1064.5361 | 0.704227 | Non-Toxin |
| 29 | NPSGPGGPTGKDGAGR | 1423.6804 | 0.701498 | Non-Toxin |
| 30 | NPAGPAGPRGPSGE | 1262.6003 | 0.695935 | Non-Toxin |
| 31 | FGPGADNAGGK | 989.4566 | 0.690454 | Non-Toxin |
| 32 | QAGPAGPR | 752.3929 | 0.683607 | Non-Toxin |
| 33 | GPAGPRGPS | 794.4034 | 0.681857 | Non-Toxin |
| 34 | SGPAGPRGPSGS | 1025.489 | 0.679261 | Non-Toxin |
| 35 | KAPDPLRGGHY | 1209.6255 | 0.673412 | Non-Toxin |
| 36 | AHGPSGPRGPAGPH | 1293.6326 | 0.666935 | Non-Toxin |
| 37 | QAGPAGPRGSPGAA | 1192.5947 | 0.665539 | Non-Toxin |
| 38 | QAGPAGPRGSPGA | 1121.5576 | 0.66391 | Non-Toxin |
| 39 | AAGPSGPRGPA | 936.4777 | 0.66159 | Non-Toxin |
| 40 | TGGPQGGLGPR | 1035.5461 | 0.661556 | Non-Toxin |
| 41 | GAHGPSGRPGPAGPH | 1350.6541 | 0.654835 | Non-Toxin |
| 42 | DFLLK | 634.369 | 0.654617 | Non-Toxin |
| 43 | GPMGPRGPGPPPSSGPQ | 1644.7678 | 0.649798 | Toxin |
| 44 | NGGPSGPR | 740.3565 | 0.648295 | Non-Toxin |
| 45 | GPAGPRGPSGE | 980.4675 | 0.644932 | Non-Toxin |
| 46 | LDMLGPR | 816.4164 | 0.643629 | Non-Toxin |

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|----|------------------|-----------|----------|-----------|
| 47 | EAPDPLRN | 910.4508 | 0.640178 | Non-Toxin |
| 48 | GPSGPQGSRGEPGPN | 1392.6382 | 0.63726 | Non-Toxin |
| 49 | NVGEPGRLF | 987.5137 | 0.627502 | Non-Toxin |
| 50 | NNPDPLRGGHY | 1238.5791 | 0.626809 | Non-Toxin |
| 51 | QPPGPTGAP | 820.4079 | 0.620057 | Non-Toxin |
| 52 | KAPDPLRNHY | 1209.6255 | 0.616423 | Non-Toxin |
| 53 | QHGGPSRPGGAGHP | 1407.6755 | 0.613812 | Non-Toxin |
| 54 | GPAGVRGPAGPA | 1005.5355 | 0.610344 | Non-Toxin |
| 55 | GPAGPRGSPGSN | 1052.4998 | 0.591846 | Non-Toxin |
| 56 | PAGGSPRGPA | 865.4406 | 0.582409 | Non-Toxin |
| 57 | GPAGPRGSPGS | 938.457 | 0.581542 | Non-Toxin |
| 58 | QPVGEPGKGAGSPSGE | 1606.7585 | 0.571822 | Non-Toxin |
| 59 | EAPDPLRNHY | 1210.573 | 0.554447 | Non-Toxin |
| 60 | QPGTAAGPGR | 910.462 | 0.552907 | Non-Toxin |
| 61 | GPVGEPGKQGSPSGE | 1535.7214 | 0.550743 | Non-Toxin |
| 62 | NAPGPGGPTGKDGARQ | 1478.7227 | 0.534334 | Non-Toxin |
| 63 | QPVGEPGKGAGPSGPS | 1420.6946 | 0.520827 | Non-Toxin |
| 64 | NPSGPGGPTGKDAGQR | 1494.7175 | 0.505074 | Non-Toxin |
| 65 | AGPVGEPGKGA | 938.4821 | 0.503477 | Non-Toxin |
| 66 | VGAGPSGPR | 796.4191 | 0.490303 | Non-Toxin |
| 67 | TSQFADRL | 936.4665 | 0.484124 | Non-Toxin |
| 68 | KLPALPAK | 836.5483 | 0.470238 | Non-Toxin |
| 69 | NPAGPRGPSGE | 1037.489 | 0.469391 | Non-Toxin |
| 70 | QGGVGELPGR | 968.5039 | 0.469341 | Non-Toxin |
| 71 | QPTAPDGQPGAK | 1165.5728 | 0.467289 | Non-Toxin |
| 72 | AGPRGPDSGPSQR | 1280.6221 | 0.465836 | Non-Toxin |
| 73 | DDVVRF | 749.3708 | 0.464564 | Non-Toxin |
| 74 | SGLDGAKGDSGPAGPK | 1412.6895 | 0.461318 | Non-Toxin |
| 75 | FGGRGEPGPAQR | 1227.6108 | 0.458999 | Non-Toxin |
| 76 | HGPVGKDHS GPGR | 1299.6431 | 0.451958 | Non-Toxin |
| 77 | GLDGAKGDSGPAGPK | 1325.6575 | 0.442598 | Non-Toxin |
| 78 | GPVGKDGRGPAH | 1146.5894 | 0.439001 | Non-Toxin |
| 79 | QNAPGPGGPTGKDQRQ | 1606.7812 | 0.427997 | Non-Toxin |
| 80 | SGLDGAKGDTGPAGPK | 1426.7051 | 0.427327 | Non-Toxin |
| 81 | EEAPDPLRNHY | 1339.6157 | 0.41707 | Non-Toxin |

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|-----|---------------------|-----------|----------|-----------|
| 82 | GPVGNTGPK | 825.4344 | 0.4108 | Non-Toxin |
| 83 | GPHGVPGKD | 862.4297 | 0.409573 | Non-Toxin |
| 84 | NGVGELPGR | 897.4668 | 0.402994 | Non-Toxin |
| 85 | LDGAKGDSGPAGPK | 1268.636 | 0.397137 | Non-Toxin |
| 86 | RGTGPVGMSPGR | 1170.5928 | 0.381551 | Non-Toxin |
| 87 | DAGKTLPR | 856.4766 | 0.378726 | Non-Toxin |
| 88 | KGEPGHHKGPDRHTSDGPR | 1926.9409 | 0.375891 | Non-Toxin |
| 89 | DGAKGDTGPAGPK | 1169.5676 | 0.374624 | Non-Toxin |
| 90 | VRPPAGK | 723.4391 | 0.356249 | Non-Toxin |
| 91 | RPPAGKD | 739.3976 | 0.355871 | Non-Toxin |
| 92 | RNGGPEGPAGAR | 1137.564 | 0.352671 | Non-Toxin |
| 93 | LLAPPER | 794.465 | 0.351403 | Non-Toxin |
| 94 | LDGAKGDTGPAGPK | 1282.6516 | 0.344193 | Non-Toxin |
| 95 | VSPDMTSLPTLPK | 1400.7222 | 0.332511 | Non-Toxin |
| 96 | RNGPEGPAGAR | 1080.5425 | 0.330769 | Non-Toxin |
| 97 | VKLPAIPAK | 935.6168 | 0.319142 | Non-Toxin |
| 98 | TEPVLPPSKSL | 1166.6545 | 0.317239 | Non-Toxin |
| 99 | DRGKGEPGAAGPK | 1238.6367 | 0.315499 | Non-Toxin |
| 100 | LLAPPERKY | 1085.6233 | 0.312105 | Non-Toxin |
| 101 | EGPKGNGRGETGPA | 1268.6108 | 0.307589 | Non-Toxin |
| 102 | VVRPPAGK | 822.5075 | 0.298109 | Non-Toxin |
| 103 | AVLLPK | 639.4319 | 0.297171 | Non-Toxin |
| 104 | SLGGASGTSAMKQ | 1209.5659 | 0.296767 | Non-Toxin |
| 105 | LEEPGLR | 812.4391 | 0.294498 | Non-Toxin |
| 106 | VRPAPGKD | 838.4661 | 0.286871 | Non-Toxin |
| 107 | TGPAGRSPDAGAA | 1126.5366 | 0.286774 | Non-Toxin |
| 108 | GEEGKRGPTGELG | 1285.6262 | 0.261458 | Non-Toxin |
| 109 | DVSGGYDEYR | 1159.478 | 0.258236 | Non-Toxin |
| 110 | DLGDDKVRLS | 1116.5774 | 0.257807 | Non-Toxin |
| 111 | NPVGNTGPKGA | 1010.5145 | 0.253624 | Non-Toxin |
| 112 | LDPDTAYK | 921.4443 | 0.250948 | Non-Toxin |
| 113 | VAPLDHVV | 912.4705 | 0.246187 | Non-Toxin |
| 114 | LLAPPERKYS | 1172.6553 | 0.239179 | Non-Toxin |
| 115 | AKDPQSDTGTVGR | 1330.6477 | 0.237518 | Non-Toxin |
| 116 | TSPVGPTGKP | 939.5025 | 0.234554 | Non-Toxin |

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|-----|-------------|-----------|-----------|-----------|
| 117 | TEAPLNPK | 868.4654 | 0.220992 | Non-Toxin |
| 118 | VLGLGTR | 714.4388 | 0.20242 | Non-Toxin |
| 119 | RVRPPAGKD | 994.5671 | 0.195985 | Non-Toxin |
| 120 | VVRPPAGKD | 937.5345 | 0.195649 | Non-Toxin |
| 121 | KVLPVGKY | 902.5589 | 0.177888 | Non-Toxin |
| 122 | LPDTPKE | 798.4123 | 0.172989 | Non-Toxin |
| 123 | VLLDR | 614.3751 | 0.161797 | Non-Toxin |
| 124 | LDGYPKPVKEE | 1273.6553 | 0.158243 | Non-Toxin |
| 125 | LDLVDR | 729.4021 | 0.156631 | Non-Toxin |
| 126 | SVDDGKDRVS | 1076.5098 | 0.142525 | Non-Toxin |
| 127 | TDLPKVQ | 799.444 | 0.0882873 | Non-Toxin |
| 128 | TVPTLPKET | 984.5491 | 0.0878987 | Non-Toxin |
| 129 | ELTDLPKVQ | 1041.5706 | 0.0848902 | Non-Toxin |
| 130 | LTGEPHAV | 909.4556 | 0.0825041 | Non-Toxin |
| 131 | REVLDSVR | 972.5352 | 0.0754738 | Non-Toxin |

Table S2. Results of TSP-II screened by ToxinPred and PeptideRanker.

| NO. | Peptide | Length | Mass (Da) | ToxinPred | Peptide Ranker Score |
|-----|----------------------|--------|-----------|-----------|----------------------|
| 1 | GPAGGPLGPR | 10 | 877.48 | Non-Toxin | 0.8998 |
| 2 | AGPGAGPLGPR | 11 | 948.51 | Non-Toxin | 0.8988 |
| 3 | GPAGPAGPGPPSGR | 15 | 1230.61 | Non-Toxin | 0.8858 |
| 4 | AGPRGPAGPSGLR | 13 | 1191.65 | Non-Toxin | 0.8848 |
| 5 | DFCPPGFNTK | 10 | 1181.52 | Non-Toxin | 0.8842 |
| 6 | QGPAGGPLGPR | 11 | 1005.54 | Non-Toxin | 0.8801 |
| 7 | KAPDPF | 6 | 673.34 | Non-Toxin | 0.8677 |
| 8 | SGPAGPAGPGPPRQ | 15 | 1301.65 | Non-Toxin | 0.8619 |
| 9 | QGSGPAGPAGPGPPQR | 17 | 1486.73 | Non-Toxin | 0.8413 |
| 10 | SDFPALR | 7 | 804.41 | Non-Toxin | 0.8326 |
| 11 | AGPEGPLGPR | 10 | 949.50 | Non-Toxin | 0.8115 |
| 12 | AGPSGPAGRP | 10 | 865.44 | Non-Toxin | 0.8080 |
| 13 | QGGGPQGPVGNTGPKG | 16 | 1406.69 | Non-Toxin | 0.7910 |
| 14 | GPAGPAGRPGPSGE | 14 | 1205.58 | Non-Toxin | 0.7842 |
| 15 | VFPGLPDHL | 9 | 993.53 | Non-Toxin | 0.7765 |
| 16 | GPAGPRGPA | 9 | 778.41 | Non-Toxin | 0.7621 |
| 17 | PGPSGPKGEPGAK | 13 | 1177.61 | Non-Toxin | 0.7621 |
| 18 | GPPGTPGSPGPPGFEGGKPQ | 20 | 1816.87 | Non-Toxin | 0.7609 |
| 19 | NSAGPQGGLGPR | 12 | 1149.59 | Non-Toxin | 0.7514 |
| 20 | GPAGPRSGPGAA | 12 | 993.50 | Non-Toxin | 0.7429 |
| 21 | GPSGPRGPA | 9 | 794.40 | Non-Toxin | 0.7422 |
| 22 | QHGPSGPRGPAGPHGPVGKD | 20 | 1903.94 | Non-Toxin | 0.7407 |

| | | | | | |
|----|------------------|----|---------|-----------|--------|
| 23 | GPRGPAGPHGPPVVKD | 15 | 1397.72 | Non-Toxin | 0.7323 |
| 24 | GPSGRPGPAGPH | 12 | 1085.54 | Non-Toxin | 0.7228 |
| 25 | AGPAGPRGSPGA | 12 | 993.50 | Non-Toxin | 0.7158 |
| 26 | AGPRGASGPAGPR | 13 | 1149.60 | Non-Toxin | 0.7083 |
| 27 | LGPAGPR | 7 | 666.38 | Non-Toxin | 0.7051 |
| 28 | AGPAGPRGSPGAA | 13 | 1064.54 | Non-Toxin | 0.7042 |
| 29 | NPSGPGGPTGKDGAGR | 16 | 1423.68 | Non-Toxin | 0.7015 |
| 30 | NPAGPAGPRGPSGE | 14 | 1262.60 | Non-Toxin | 0.6959 |
| 31 | FGPGADNAGGK | 11 | 989.46 | Non-Toxin | 0.6905 |
| 32 | QAGPAGPR | 8 | 752.39 | Non-Toxin | 0.6836 |
| 33 | GPAGPRGPS | 9 | 794.40 | Non-Toxin | 0.6819 |
| 34 | SGPAGPRGPSGS | 12 | 1025.49 | Non-Toxin | 0.6793 |
| 35 | KAPDPLRGHGY | 11 | 1209.63 | Non-Toxin | 0.6734 |
| 36 | AHGSPGPRGPAGPH | 14 | 1293.63 | Non-Toxin | 0.6669 |
| 37 | QAGPAGPRGSPGAA | 14 | 1192.59 | Non-Toxin | 0.6655 |
| 38 | QAGPAGPRGSPGA | 13 | 1121.56 | Non-Toxin | 0.6639 |
| 39 | AAGPSGPRGPA | 11 | 936.48 | Non-Toxin | 0.6616 |
| 40 | TGGPQGPLGPR | 11 | 1035.55 | Non-Toxin | 0.6616 |
| 41 | GAHGSPGRPGPAGPH | 15 | 1350.65 | Non-Toxin | 0.6548 |
| 42 | DFLK | 5 | 634.37 | Non-Toxin | 0.6546 |
| 43 | NGGPSGPR | 8 | 740.36 | Non-Toxin | 0.6483 |
| 44 | GPAGPRGPSGE | 11 | 980.47 | Non-Toxin | 0.6449 |
| 45 | LDMLGPR | 7 | 816.42 | Non-Toxin | 0.6436 |
| 46 | EAPDPLRN | 8 | 910.45 | Non-Toxin | 0.6402 |
| 47 | GPSGPQGSRGEPGPN | 15 | 1392.64 | Non-Toxin | 0.6373 |
| 48 | NVGEPGRLF | 9 | 987.51 | Non-Toxin | 0.6275 |
| 49 | NNPDPLRGHGY | 11 | 1238.58 | Non-Toxin | 0.6268 |
| 50 | QPPGPTGAP | 9 | 820.41 | Non-Toxin | 0.6201 |
| 51 | KAPDPLRNHY | 10 | 1209.63 | Non-Toxin | 0.6164 |
| 52 | QHGGSPRPGAGHP | 15 | 1407.68 | Non-Toxin | 0.6138 |
| 53 | GPAGVRGPAGPA | 12 | 1005.54 | Non-Toxin | 0.6103 |

Table S3. Results of TSP-II screened by AIPpred and AllerTOP 2.0.

| NO. | Peptide | Length | Mass (Da) | AIP or Non-AIP | AIP Score | Allergenicity |
|-----|------------|--------|-----------|----------------|-----------|---------------|
| 1 | DFLLK | 5 | 634.369 | AIP | 0.6023 | Yes |
| 2 | AGPEGPLGPR | 10 | 949.4981 | AIP | 0.4767 | Yes |
| 3 | DFCPPGFNTK | 10 | 1181.518 | AIP | 0.4651 | No |
| 4 | NVGEPGRLF | 9 | 987.5137 | AIP | 0.4581 | Yes |
| 5 | EAPDPLRN | 8 | 910.4508 | AIP | 0.4558 | No |
| 6 | SDFPALR | 7 | 804.413 | AIP | 0.4465 | Yes |
| 7 | GPAGPRGPS | 9 | 794.4034 | AIP | 0.4372 | Yes |
| 8 | AGPSGPAGRP | 10 | 865.4406 | AIP | 0.4349 | Yes |
| 9 | GPAGGPLGPR | 10 | 877.4769 | AIP | 0.4349 | Yes |
| 10 | KAPDPLRNHY | 10 | 1209.626 | AIP | 0.4326 | Yes |
| 11 | KAPDPF | 6 | 673.3435 | AIP | 0.4209 | Yes |

| | | | | | | |
|----|-----------|---|----------|---------|--------|-----|
| 12 | LGPAGPR | 7 | 666.3813 | AIP | 0.4116 | Yes |
| 13 | GPAGPRGPA | 9 | 778.4085 | AIP | 0.3977 | Yes |
| 14 | QAGPAGPR | 8 | 752.3929 | AIP | 0.3907 | Yes |
| 15 | GPSGPRGPA | 9 | 794.4034 | AIP | 0.3791 | Yes |
| 16 | NGGPSGPR | 8 | 740.3565 | AIP | 0.3767 | No |
| 17 | LDMLGPR | 7 | 816.4164 | AIP | 0.3605 | Yes |
| 18 | VFPGLPDHL | 9 | 993.5283 | Non-AIP | 0.3465 | Yes |
| 19 | QPPGPTGAP | 9 | 820.4079 | Non-AIP | 0.2512 | Yes |