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APPENDIX 1

Honey samples which were used in this study

Figure 27: Malaysian and New Zealand honey samples which were used in this study



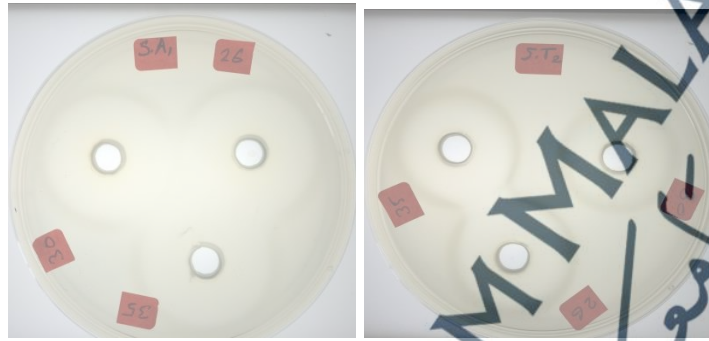
Figure 28: Libyan honey samples which were used in this study



APPENDIX 2

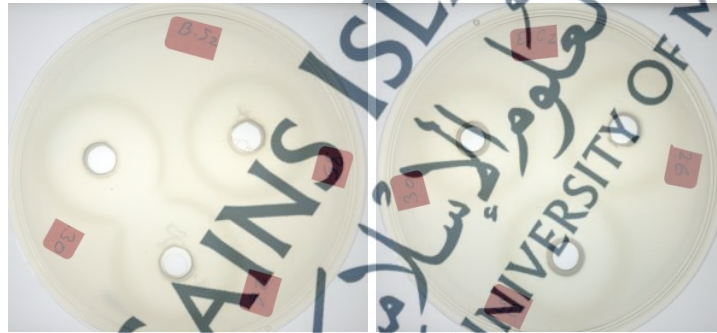
Growth inhibition zones of pathogenic bacteria using well method and their positive control

Figure 29: Growth inhibition zone of honey samples (H026, H030 and H035) against pathogenic bacteria by well method at 37°C after 24 h of Incubation



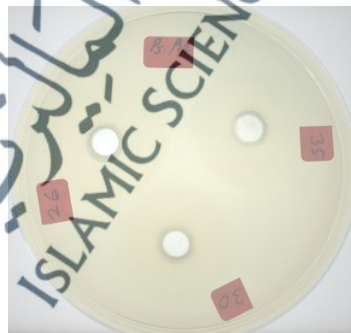
S. aureus

S. Typhimurium



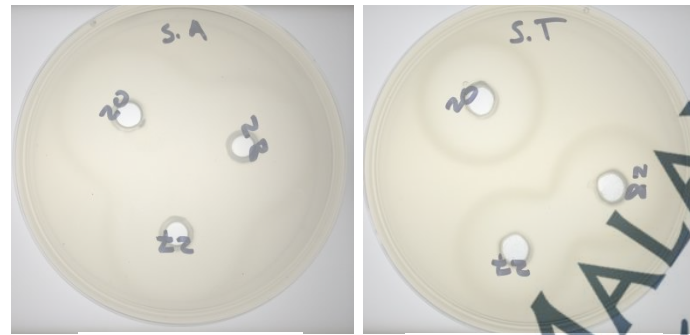
B. subtilis

E. coli



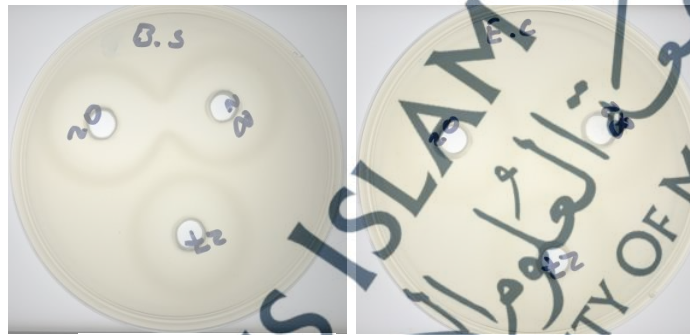
P. aeruginosa

Figure 30: Growth inhibition zone of honey samples (H020, H027 and H028) against pathogenic bacteria by well method at 37°C after 24 h of Incubation



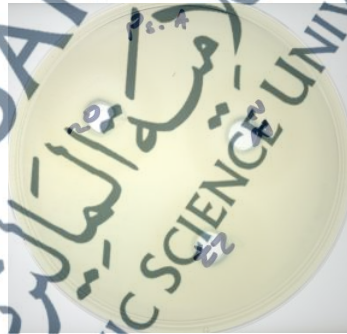
S. aureus

S. Typhimurium



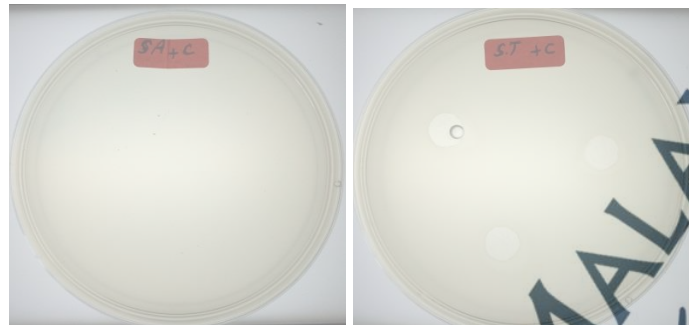
B. subtilis

E. coli



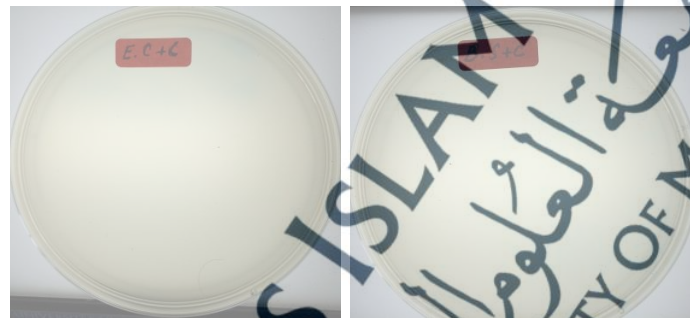
P. aeruginosa

Figure 31: Growth of pathogenic bacteria on nutrient agar plates as positive control by well method at 37°C after 24 h of incubation



S. aureus

S. Typhimurium



E. coli

B. subtilis



P. aeruginosa

APPENDIX 3

Standard curves of gallic acid, BSA, glutathione and Log of MW of marker

Figure 32: Standard curve of gallic acid at OD₇₆₅ nm for freeze dried honey samples

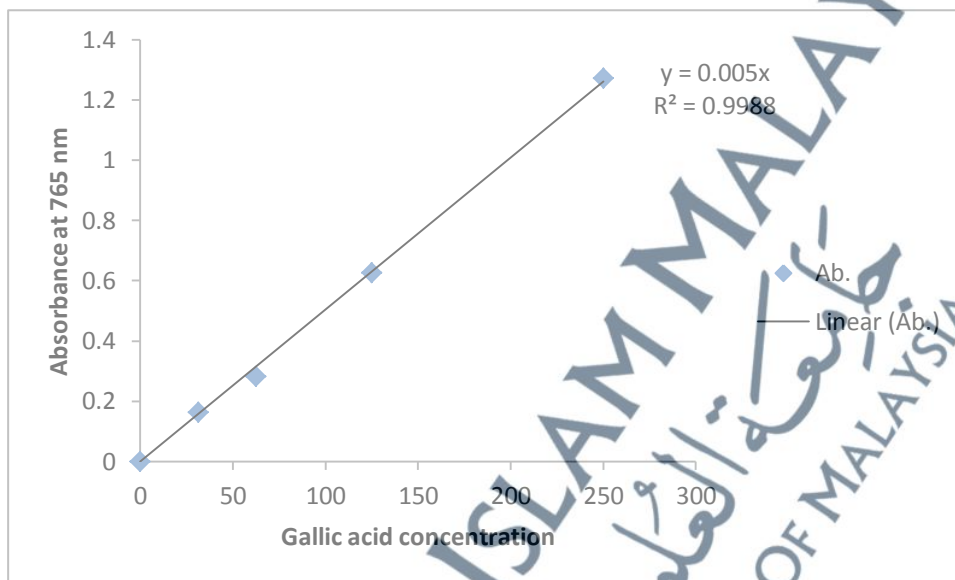


Figure 33: Standard curve of BSA between concentration and absorbance for freeze dried honey samples

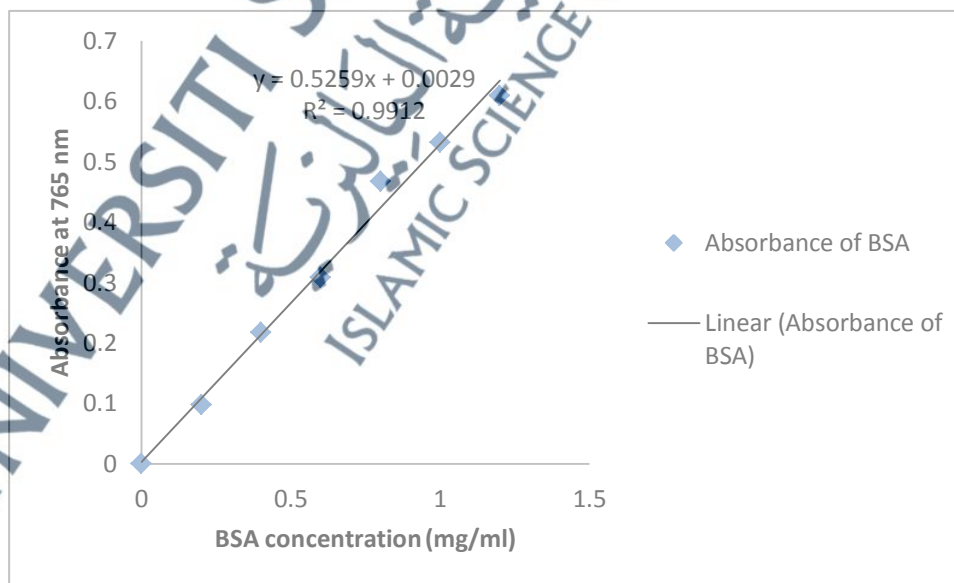


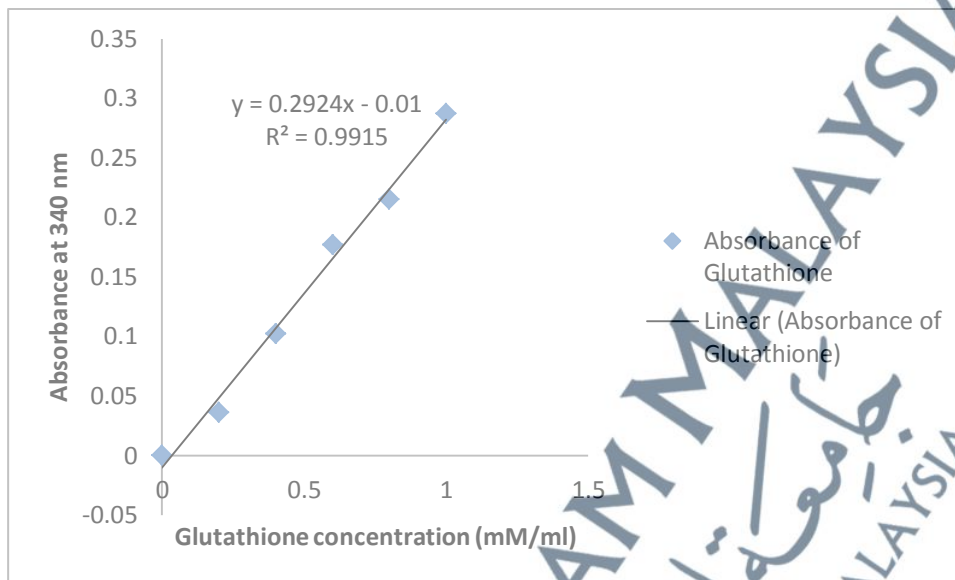
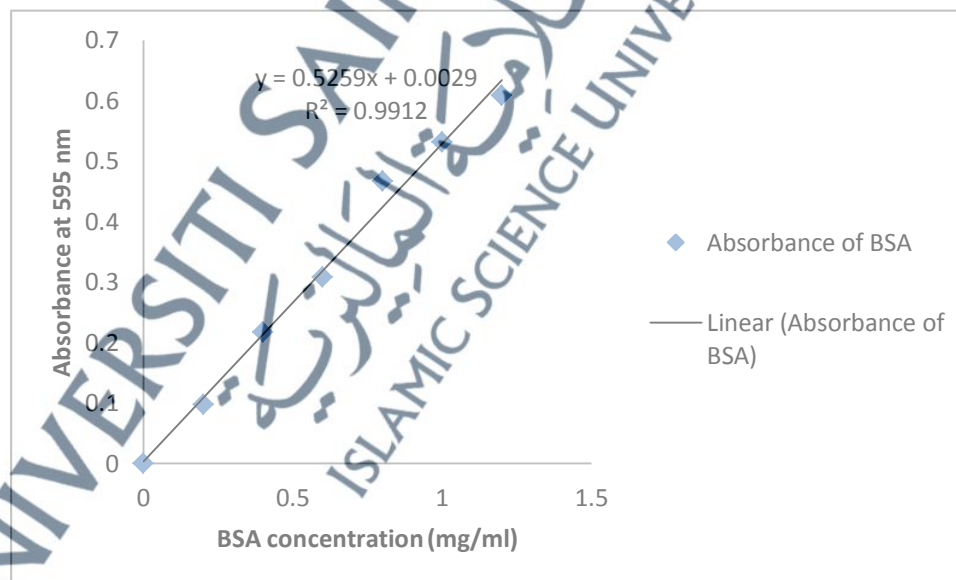
Figure 34: Standard curve of glutathione for freeze dried honey samples**Figure 35:** Standard curve of BSA between concentration and absorbance for freeze dried extracted proteins

Figure 36: Standard curve of glutathione between concentration and absorbance for freeze dried extracted proteins

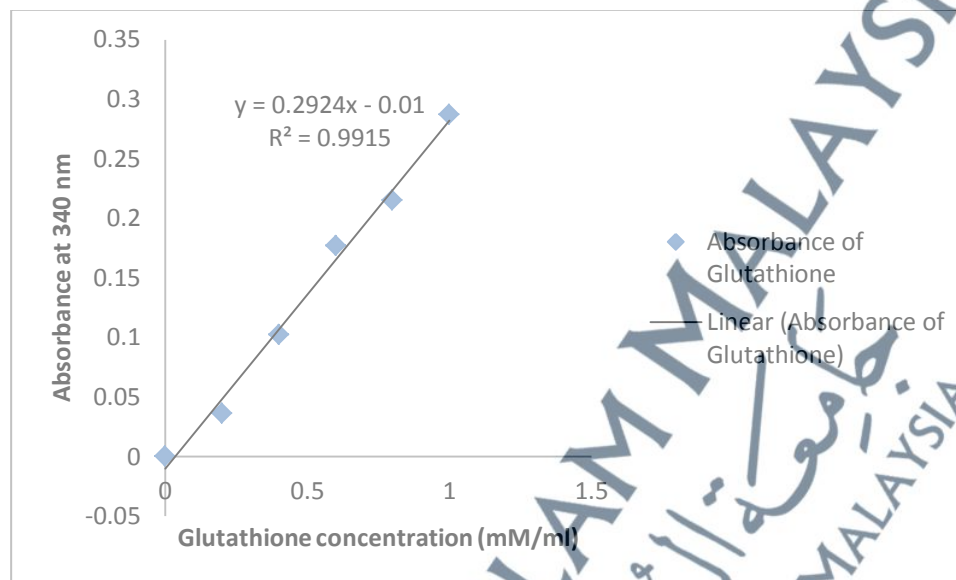


Figure 37: Standard curve of glutathione between concentration and absorbance for RP-HPLC fractions

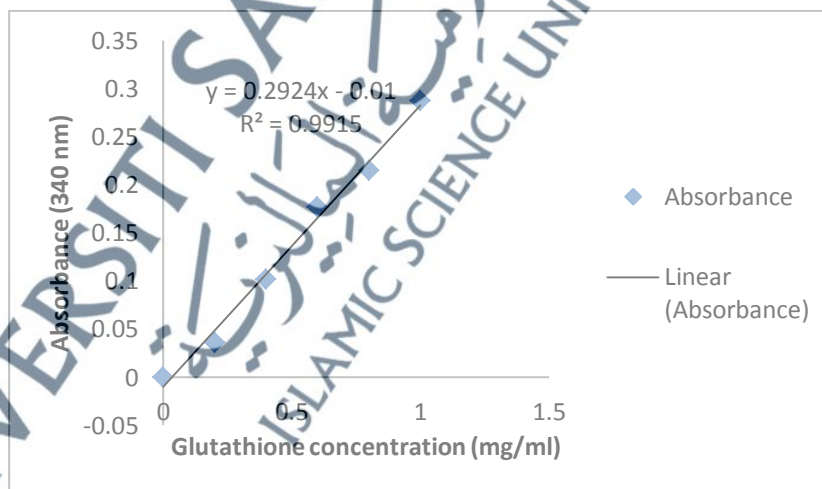


Figure 38: Standard curve of glutathione between concentration and absorbance for Sephadex G-50 Fine fractions

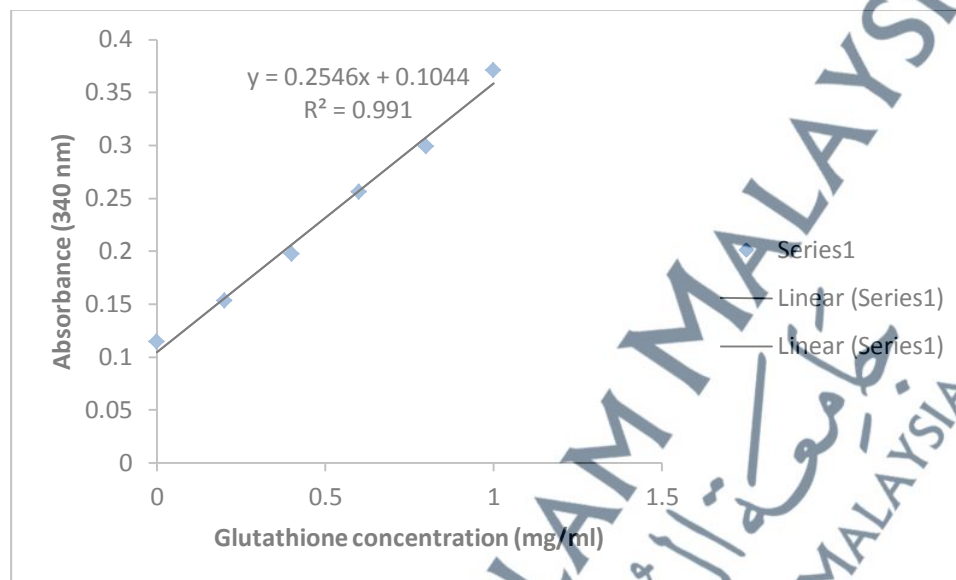
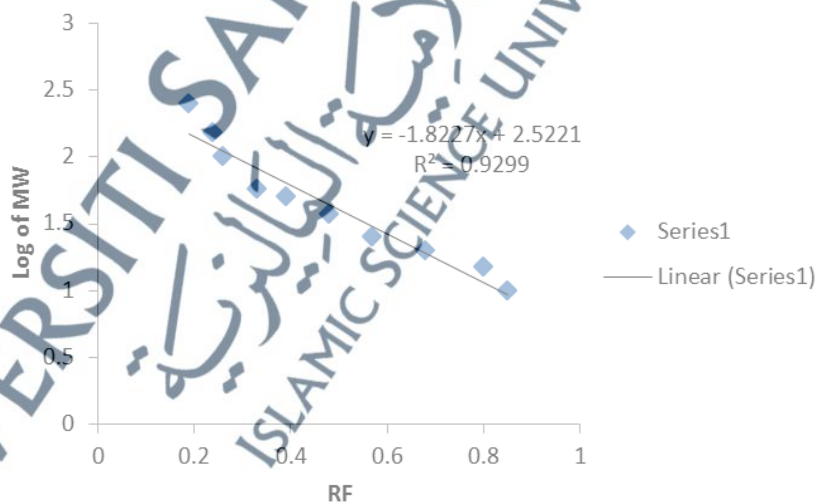


Figure 39: Standard curve of Log of MW of marker of SDS-Page with R_f



APPENDIX 4

Amino acid curves of honey samples precipitated proteins

Figure 40: Amino acid separation curve of precipitated proteins from honey samples H020 (Hanoon honey) using HPLC

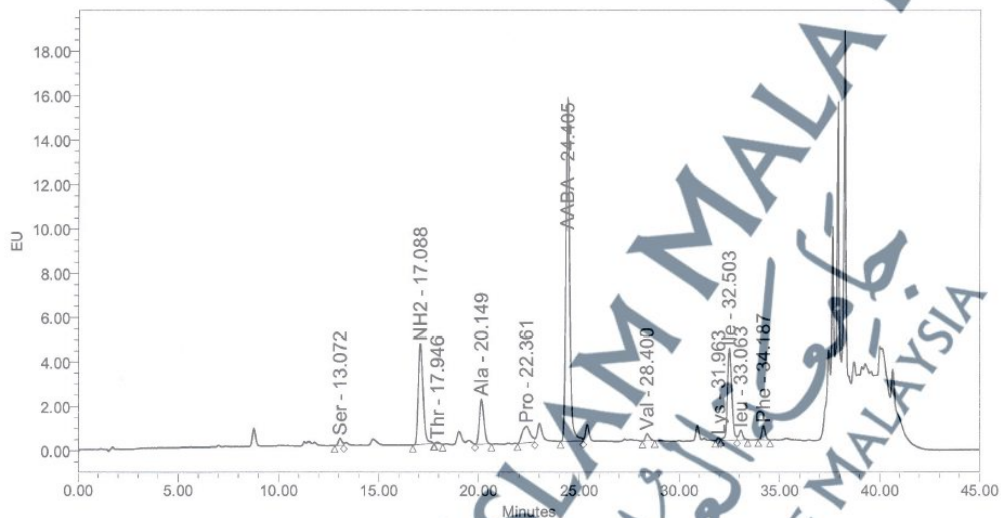


Figure 41: Amino acid separation curve of precipitated proteins from honey samples H025 (Alder honey) using HPLC

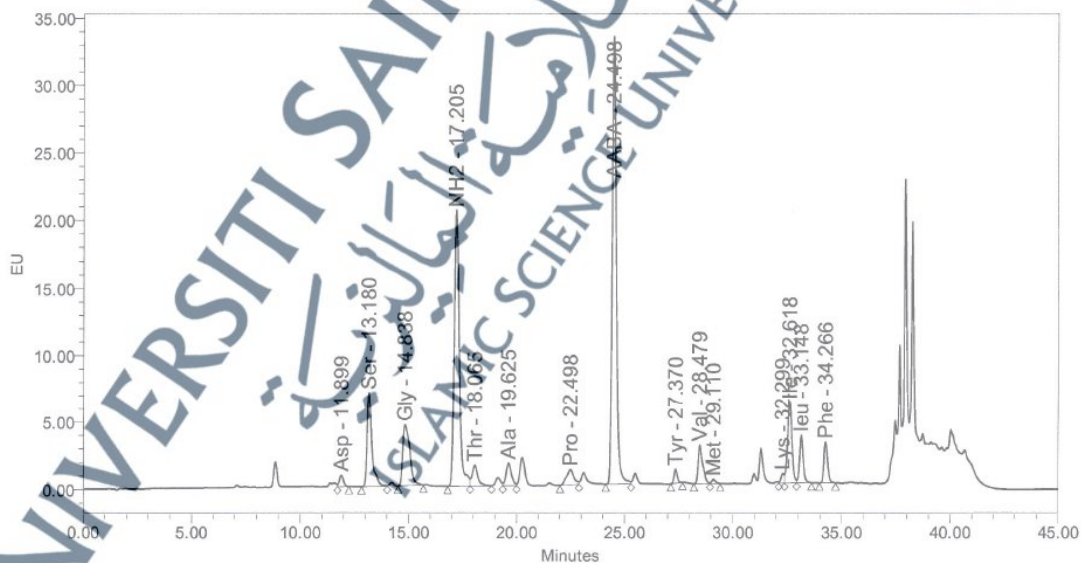


Figure 42: Amino acid separation curve of precipitated proteins from honey samples H026 (Tualang honey) using HPLC

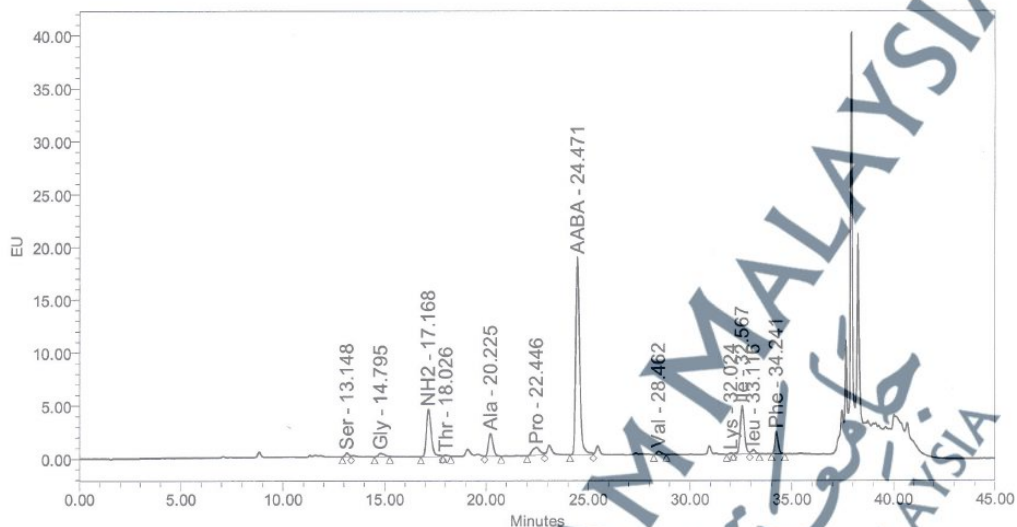


Figure 43: Amino acid separation curve of precipitated proteins from honey samples H027 (Manuka honey) using HPLC

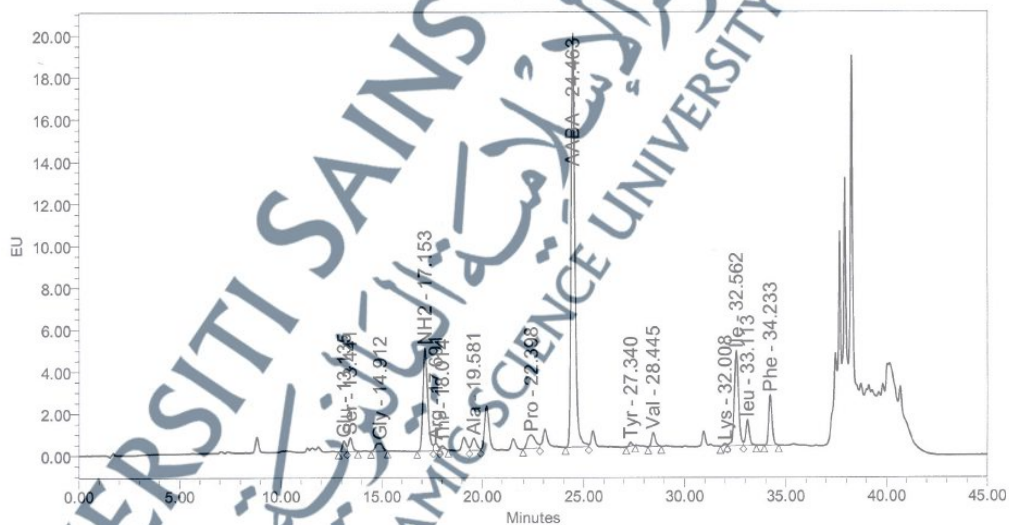


Figure 44: Amino acid separation curve of precipitated proteins from honey samples H028 (Kharoob honey) using HPLC

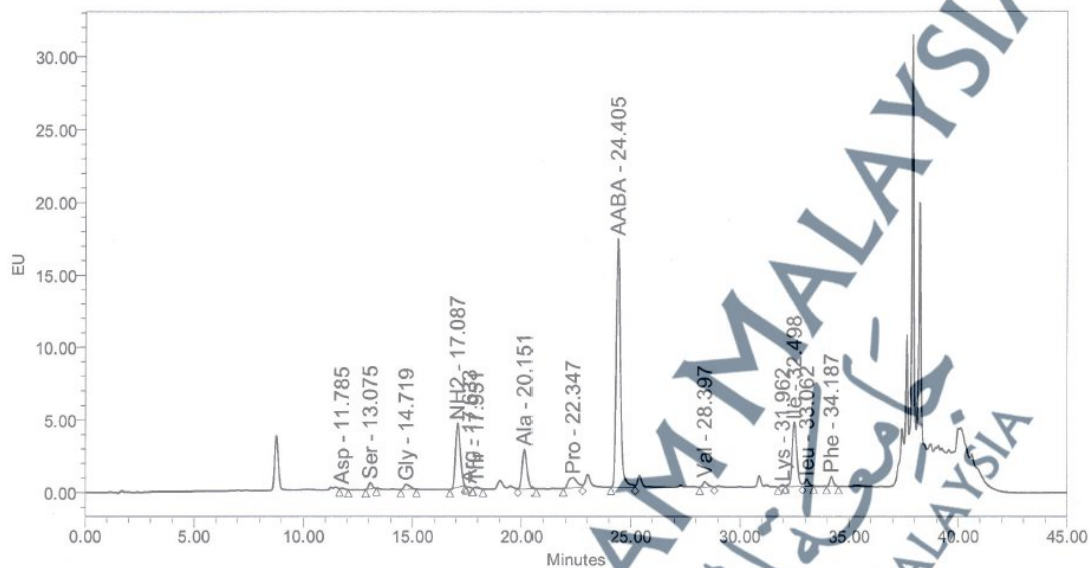


Figure 45: Amino acid separation curve of precipitated proteins from honey samples H030 (Acacia honey) using HPLC

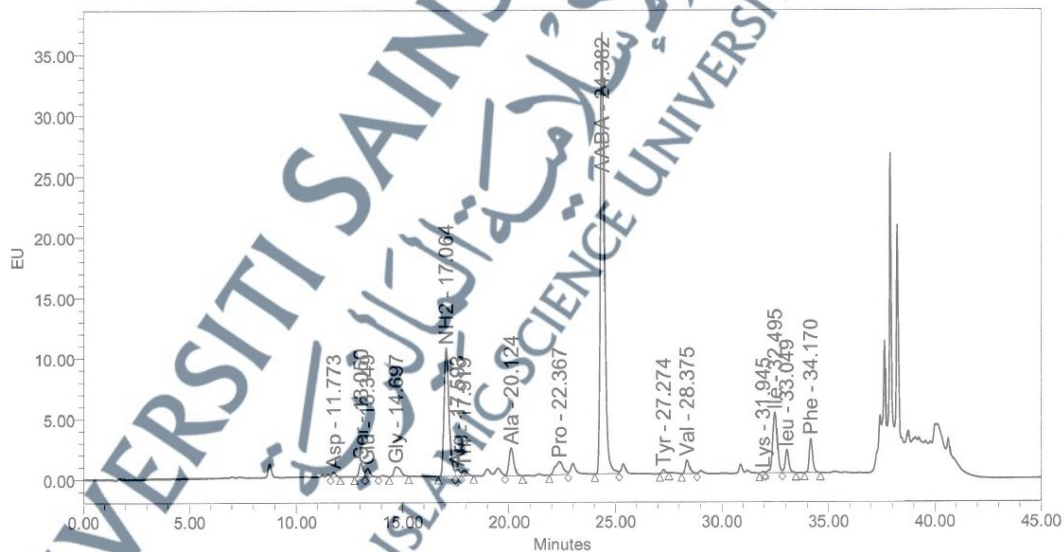


Figure 46: Amino acid separation curve of precipitated proteins from honey samples H031 (Acacia honey) using HPLC

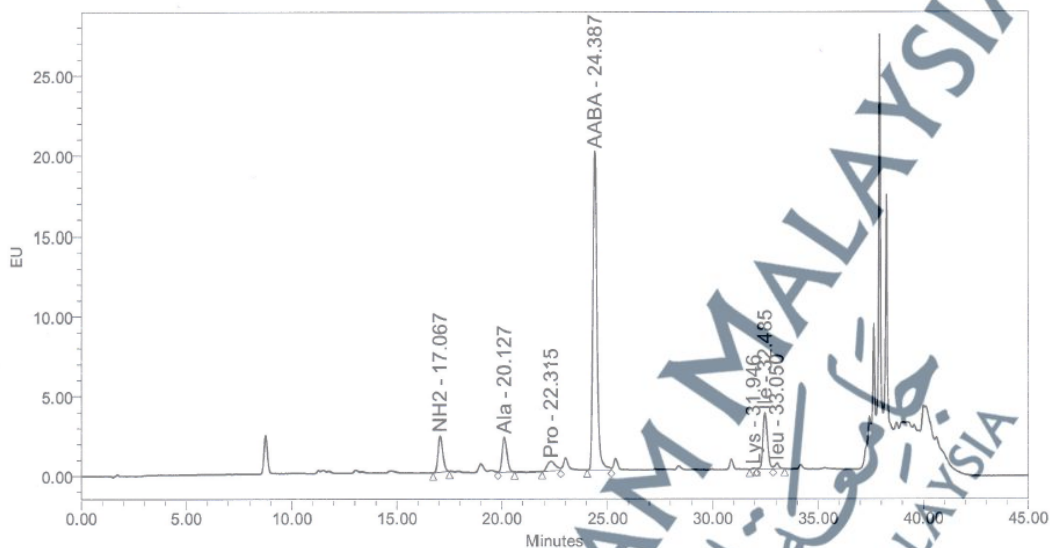


Figure 47: Amino acid separation curve of precipitated proteins from honey samples H032 (Acacia honey) using HPLC

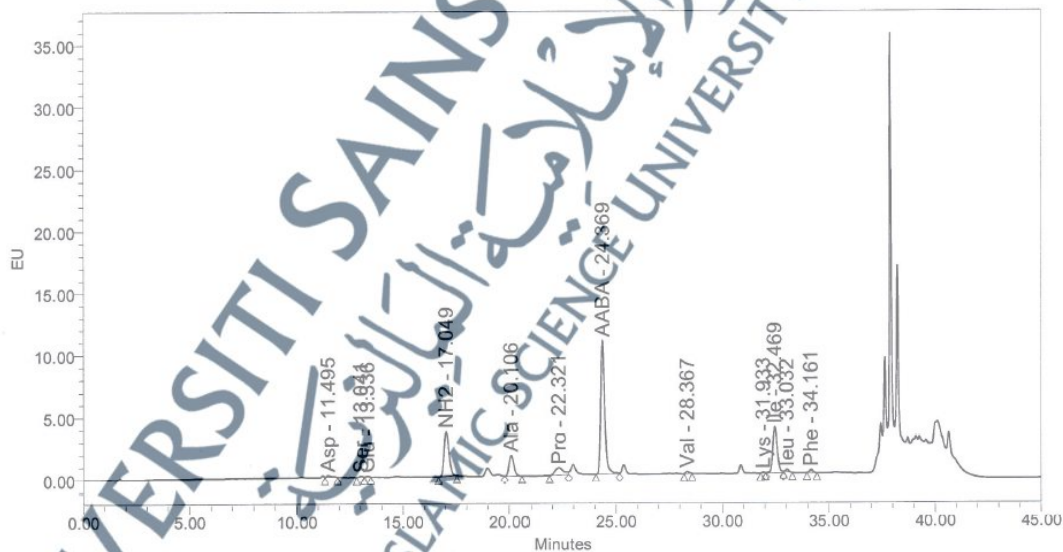
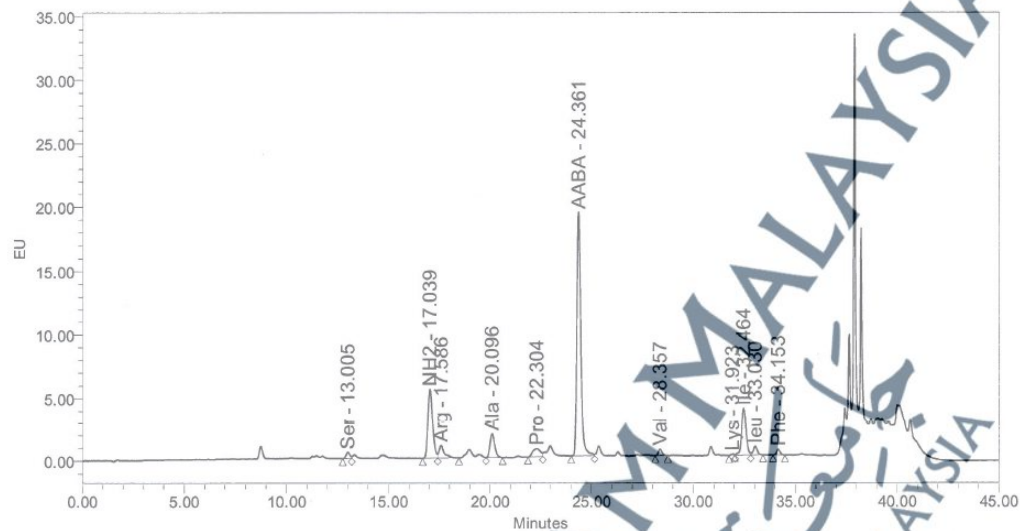


Figure 48: Amino acid separation curve of precipitated proteins from honey samples H035 (Manuka honey) using HPLC



Appendix 5

Information of RP-HPLC fractions from precipitated proteins from honey samples

Table 43: RP-HPLC fractions information from precipitated proteins from honey sample H025 (Alseder honey)

```

=====
Fraction collection using a timetable
=====
Frac Well Location Volume BeginTime EndTime Reason Mass
# # # [µl] [min] [min]
-----|-----|-----|-----|-----|-----|-----
1 1 1-Vial 1 5994.17 0.0015 1.5001 Time
2 1 1-Vial 2 5956.67 1.5109 3.0001 Time
3 1 1-Vial 3 5960.83 3.0099 4.5001 Time
4 1 1-Vial 4 5958.33 4.5105 6.0001 Time
5 1 1-Vial 5 5956.67 6.0113 7.5005 Time
6 1 1-Vial 6 5896.67 7.5259 9.0001 Time
7 1 1-Vial 7 5960.83 9.0099 10.5001 Time
8 1 1-Vial 8 5955.00 10.5113 12.0001 Time
9 1 1-Vial 9 5960.83 12.0099 13.5001 Time
10 1 1-Vial 10 5958.33 13.5107 15.0003 Time
11 1 1-Vial 11 5890.00 15.0276 16.5001 Time
12 1 1-Vial 12 5956.67 16.5109 18.0001 Time
13 1 1-Vial 13 5960.83 18.0099 19.5001 Time
14 1 1-Vial 14 5960.83 19.5099 21.0001 Time
15 1 1-Vial 15 5961.67 21.0099 22.5003 Time
16 1 1-Vial 101 5903.33 22.5243 24.0001 Time

17 1 1-Vial 102 5960.83 24.0099 25.5001 Time
18 1 1-Vial 103 5960.83 25.5099 27.0001 Time
19 1 1-Vial 104 5960.00 27.0001 28.5001 Time
20 1 1-Vial 105 5961.67 28.5099 30.0003 Time
21 1 1-Vial 106 5967.50 30.0282 31.5001 Time
22 1 1-Vial 107 5960.83 31.5099 33.0001 Time
23 1 1-Vial 108 5955.00 33.0113 34.5001 Time
24 1 1-Vial 109 5956.67 34.5109 36.0001 Time
25 1 1-Vial 110 5960.83 36.0101 37.5003 Time
26 1 1-Vial 111 5896.67 37.5261 39.0003 Time
27 1 1-Vial 112 5990.00 39.0526 40.5001 Time
28 1 1-Vial 113 5960.83 40.5099 42.0001 Time
29 1 1-Vial 114 5956.67 42.0109 43.5001 Time
30 1 1-Vial 115 5960.83 43.5103 45.0003 Time
31 1 1-Vial 201 5901.67 45.0247 46.5001 Time
32 1 1-Vial 202 5960.83 46.5099 48.0001 Time
33 1 1-Vial 203 5960.83 48.0099 49.5001 Time
34 1 1-Vial 204 5957.50 49.5107 51.0001 Time
35 1 1-Vial 205 5961.67 51.0099 52.5003 Time
36 1 1-Vial 206 5909.17 52.5228 54.0001 Time
37 1 1-Vial 207 5960.83 54.0099 55.5001 Time
38 1 1-Vial 208 5958.33 55.5105 57.0001 Time
39 1 1-Vial 209 5960.83 57.0099 58.5001 Time
40 1 1-Vial 210 5960.83 58.5101 60.0003 Time
41 1 1-Vial 211 5887.50 60.0282 61.5001 Time
42 1 1-Vial 212 5956.67 61.5109 63.0001 Time
43 1 1-Vial 213 5960.83 63.0099 64.5001 Time
44 1 1-Vial 214 5957.50 64.5107 66.0001 Time
45 1 1-Vial 215 5443.33 66.0099 67.3707 Time
=====

```

Table 44: RP-HPLC fractions information from precipitated proteins from honey sample H028 (Alseder honey)

```

=====
Fraction collection using a timetable
=====
Frac Well Location Volume BeginTime EndTime Reason Mass
# # [µl] [min] [min]
-----|-----|-----|-----|-----|-----|-----
1 1 1-Vial 1 5994.17 0.0015 1.5001 Time
2 1 1-Vial 2 5953.33 1.5118 3.0001 Time
3 1 1-Vial 3 5960.83 3.0099 4.5001 Time
4 1 1-Vial 4 5960.00 4.5101 6.0001 Time
5 1 1-Vial 5 5960.83 6.0101 7.5003 Time
6 1 1-Vial 6 5890.83 7.5274 9.0001 Time
7 1 1-Vial 7 5960.83 9.0099 10.5001 Time
8 1 1-Vial 8 5959.17 10.5103 12.0001 Time
9 1 1-Vial 9 5960.83 12.0099 13.5001 Time
10 1 1-Vial 10 5960.83 13.5101 15.0003 Time
11 1 1-Vial 11 5883.33 15.0292 16.5001 Time
12 1 1-Vial 12 5960.00 16.5101 18.0001 Time
13 1 1-Vial 13 5960.83 18.0099 19.5001 Time
14 1 1-Vial 14 5960.00 19.5101 21.0001 Time
15 1 1-Vial 15 5961.67 21.0099 22.5003 Time
16 1 1-Vial 101 5900.83 22.5249 24.0001 Time

17 1 1-Vial 102 5960.83 24.0099 25.5001 Time
18 1 1-Vial 103 5960.83 25.5099 27.0001 Time
19 1 1-Vial 104 5960.83 27.0099 28.5001 Time
20 1 1-Vial 105 5961.67 28.5099 30.0003 Time
21 1 1-Vial 106 5904.17 30.0240 31.5001 Time
22 1 1-Vial 107 5960.83 31.5099 33.0001 Time
23 1 1-Vial 108 5956.67 33.0109 34.5001 Time
24 1 1-Vial 109 5960.83 34.5099 36.0001 Time
25 1 1-Vial 110 5960.83 36.0101 37.5003 Time
26 1 1-Vial 111 5897.50 37.5259 39.0003 Time
27 1 1-Vial 112 5789.17 39.0528 40.5001 Time
28 1 1-Vial 113 5960.83 40.5099 42.0001 Time
29 1 1-Vial 114 5960.00 42.0101 43.5001 Time
30 1 1-Vial 115 5961.67 43.5099 45.0003 Time
31 1 1-Vial 201 5900.00 45.0251 46.5001 Time
32 1 1-Vial 202 5960.00 46.5101 48.0001 Time
33 1 1-Vial 203 5960.83 48.0099 49.5001 Time
34 1 1-Vial 204 5612.50 49.5101 50.9132 Time
=====

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Table 45: RP-HPLC fractions information from precipitated proteins from honey sample H026 (Alseder honey)

=====
 Fraction collection using a timetable
 =====

Frac #	Well #	Location	Volume [µl]	BeginTime [min]	EndTime [min]	Reason	Mass
1	1	1-Vial 1	5994.17	0.0015	1.5001	Time	
2	1	1-Vial 2	5956.67	1.5109	3.0001	Time	
3	1	1-Vial 3	5960.83	3.0099	4.5001	Time	
4	1	1-Vial 4	5957.50	4.5107	6.0001	Time	
5	1	1-Vial 5	5958.33	6.0109	7.5005	Time	
6	1	1-Vial 6	5889.17	7.5278	9.0001	Time	
7	1	1-Vial 7	5960.00	9.0101	10.5001	Time	
8	1	1-Vial 8	5956.67	10.5109	12.0001	Time	
9	1	1-Vial 9	5957.50	12.0107	13.5001	Time	
10	1	1-Vial 10	5958.33	13.5107	15.0003	Time	
11	1	1-Vial 11	5879.17	15.0303	16.5001	Time	
12	1	1-Vial 12	5956.67	16.5109	18.0001	Time	
13	1	1-Vial 13	5960.00	18.0101	19.5001	Time	
14	1	1-Vial 14	5960.83	19.5099	21.0001	Time	
15	1	1-Vial 15	5961.67	21.0099	22.5003	Time	
16	1	1-Vial 101	5890.83	22.5274	24.0001	Time	
17	1	1-Vial 102	5957.50	24.0107	25.5001	Time	
18	1	1-Vial 103	5960.00	25.5101	27.0001	Time	
19	1	1-Vial 104	5957.50	27.0107	28.5001	Time	
20	1	1-Vial 105	5960.83	28.5101	30.0003	Time	
21	1	1-Vial 106	5893.33	30.0268	31.5001	Time	
22	1	1-Vial 107	5960.83	31.5099	33.0001	Time	
23	1	1-Vial 108	5956.67	33.0109	34.5001	Time	
24	1	1-Vial 109	5960.00	34.5101	36.0001	Time	
25	1	1-Vial 110	5957.50	36.0109	37.5003	Time	
26	1	1-Vial 111	5887.50	37.5304	39.0003	Time	
27	1	1-Vial 112	5780.83	39.0549	40.5001	Time	
28	1	1-Vial 113	5960.00	40.5101	42.0001	Time	
29	1	1-Vial 114	5956.67	42.0109	43.5001	Time	
30	1	1-Vial 115	5960.83	43.5101	45.0003	Time	
31	1	1-Vial 201	5891.67	45.0272	46.5001	Time	
32	1	1-Vial 202	5957.50	46.5107	48.0001	Time	
33	1	1-Vial 203	5960.00	48.0101	49.5001	Time	
34	1	1-Vial 204	5956.67	49.5109	51.0001	Time	
35	1	1-Vial 205	5961.67	51.0099	52.5003	Time	
36	1	1-Vial 206	5894.17	52.5265	54.0001	Time	
37	1	1-Vial 207	5959.00	54.0101	55.5001	Time	
38	1	1-Vial 208	5957.50	55.5107	57.0001	Time	
39	1	1-Vial 209	5960.00	57.0101	58.5001	Time	
40	1	1-Vial 210	5957.50	58.5109	60.0003	Time	
41	1	1-Vial 211	5873.33	60.0318	61.5001	Time	
42	1	1-Vial 212	5957.50	61.5107	63.0001	Time	
43	1	1-Vial 213	5960.00	63.0101	64.5001	Time	
44	1	1-Vial 214	5956.67	64.5109	66.0001	Time	
45	1	1-Vial 215	5443.33	66.0099	67.3707	Time	

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Table 46: RP-HPLC fractions information from precipitated proteins from honey sample H032 (Alseder honey)

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=====
Fraction collection using a timetable
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Frac #	Well #	Location	Volume [µl]	BeginTime [min]	EndTime [min]	Reason	Mass
1	1	1-Vial 1	5994.17	0.0015	1.5001	Time	
2	1	1-Vial 2	5956.67	1.5109	3.0001	Time	
3	1	1-Vial 3	5960.83	3.0099	4.5001	Time	
4	1	1-Vial 4	5960.83	4.5099	6.0001	Time	
5	1	1-Vial 5	5958.33	6.0107	7.5003	Time	
6	1	1-Vial 6	5900.83	7.5249	9.0001	Time	
7	1	1-Vial 7	5960.83	9.0099	10.5001	Time	
8	1	1-Vial 8	5958.33	10.5105	12.0001	Time	
9	1	1-Vial 9	5960.83	12.0099	13.5001	Time	
10	1	1-Vial 10	5961.67	13.5099	15.0003	Time	
11	1	1-Vial 11	5886.67	15.0286	16.5003	Time	
12	1	1-Vial 12	5960.83	16.5099	18.0001	Time	
13	1	1-Vial 13	5960.83	18.0099	19.5001	Time	
14	1	1-Vial 14	5960.83	19.5099	21.0001	Time	
15	1	1-Vial 15	5961.67	21.0099	22.5003	Time	
16	1	1-Vial 101	5905.00	22.5238	24.0001	Time	
17	1	1-Vial 102	5961.67	24.0099	25.5003	Time	
18	1	1-Vial 103	5960.83	25.5099	27.0001	Time	
19	1	1-Vial 104	5960.83	27.0099	28.5001	Time	
20	1	1-Vial 105	5961.67	28.5099	30.0003	Time	
21	1	1-Vial 106	5908.33	30.0230	31.5001	Time	
22	1	1-Vial 107	5960.83	31.5099	33.0001	Time	
23	1	1-Vial 108	5960.00	33.0103	34.5003	Time	
24	1	1-Vial 109	5960.83	34.5099	36.0001	Time	
25	1	1-Vial 110	5960.83	36.0101	37.5003	Time	
26	1	1-Vial 111	5899.17	37.5255	39.0003	Time	
27	1	1-Vial 112	5791.67	39.0522	40.5001	Time	
28	1	1-Vial 113	5960.83	40.5099	42.0001	Time	
29	1	1-Vial 114	5960.00	42.0101	43.5001	Time	
30	1	1-Vial 115	5961.67	43.5099	45.0003	Time	
31	1	1-Vial 201	5905.00	45.0238	46.5001	Time	
32	1	1-Vial 202	5960.83	46.5099	48.0001	Time	
33	1	1-Vial 203	5960.83	48.0099	49.5001	Time	
34	1	1-Vial 204	5960.83	49.5099	51.0001	Time	
35	1	1-Vial 205	5961.67	51.0099	52.5003	Time	
36	1	1-Vial 206	5961.67	52.5222	54.0001	Time	
37	1	1-Vial 207	5960.83	54.0099	55.5001	Time	
38	1	1-Vial 208	5960.83	55.5099	57.0001	Time	
39	1	1-Vial 209	5960.83	57.0099	58.5001	Time	
40	1	1-Vial 210	5961.67	58.5099	60.0003	Time	
41	1	1-Vial 211	5885.83	60.0286	61.5001	Time	
42	1	1-Vial 212	5960.83	61.5099	63.0001	Time	
43	1	1-Vial 213	5960.83	63.0099	64.5001	Time	
44	1	1-Vial 214	5960.83	64.5099	66.0001	Time	
45	1	1-Vial 215	5443.33	66.0099	67.3707	Time	

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Table 47: RP-HPLC fractions information from precipitated proteins from honey sample H035 (Alseder honey)

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Fraction collection using a timetable

=====

Frac #	Well #	Location	Volume [µl]	BeginTime [min]	EndTime [min]	Reason	Mass
1	1	1-Vial 1	5994.17	0.0015	1.5001	Time	
2	1	1-Vial 2	5950.00	1.5126	3.0001	Time	
3	1	1-Vial 3	5960.00	3.0101	4.5001	Time	
4	1	1-Vial 4	5957.50	4.5107	6.0001	Time	
5	1	1-Vial 5	5955.00	6.0115	7.5003	Time	
6	1	1-Vial 6	5895.00	7.5263	9.0001	Time	
7	1	1-Vial 7	5960.00	9.0101	10.5001	Time	
8	1	1-Vial 8	5954.17	10.5115	12.0001	Time	
9	1	1-Vial 9	5960.00	12.0101	13.5001	Time	
10	1	1-Vial 10	5958.33	13.5107	15.0003	Time	
11	1	1-Vial 11	5886.67	15.0284	16.5001	Time	
12	1	1-Vial 12	5957.50	16.5107	18.0001	Time	
13	1	1-Vial 13	5960.00	18.0101	19.5001	Time	
14	1	1-Vial 14	5960.00	19.5101	21.0001	Time	
15	1	1-Vial 15	5960.83	21.0101	22.5003	Time	
16	1	1-Vial 101	5900.83	22.5249	24.0001	Time	
17	1	1-Vial 102	5957.50	24.0107	25.5001	Time	
18	1	1-Vial 103	5960.00	25.5101	27.0001	Time	
19	1	1-Vial 104	5957.50	27.0107	28.5001	Time	
20	1	1-Vial 105	5960.83	28.5101	30.0003	Time	
21	1	1-Vial 106	5906.67	30.0234	31.5001	Time	
22	1	1-Vial 107	5960.00	31.5101	33.0001	Time	
23	1	1-Vial 108	5953.33	33.0117	34.5001	Time	
24	1	1-Vial 109	5960.00	34.5101	36.0001	Time	
25	1	1-Vial 110	5954.17	36.0117	37.5003	Time	
26	1	1-Vial 111	5895.83	37.5263	39.0003	Time	
27	1	1-Vial 112	5957.50	39.0107	40.5001	Time	
28	1	1-Vial 113	5960.83	40.5099	42.0001	Time	
29	1	1-Vial 114	5957.50	42.0107	43.5001	Time	
30	1	1-Vial 115	5957.50	43.5109	45.0003	Time	
31	1	1-Vial 201	5895.33	45.0255	46.5001	Time	
32	1	1-Vial 202	5957.50	46.5107	48.0001	Time	
33	1	1-Vial 203	5960.00	48.0101	49.5001	Time	
34	1	1-Vial 204	5956.67	49.5109	51.0001	Time	
35	1	1-Vial 205	5960.83	51.0101	52.5003	Time	
36	1	1-Vial 206	5906.67	52.5234	54.0001	Time	
37	1	1-Vial 207	5960.83	54.0099	55.5001	Time	
38	1	1-Vial 208	5957.50	55.5107	57.0001	Time	
39	1	1-Vial 209	5957.50	57.0107	58.5001	Time	
40	1	1-Vial 210	5958.33	58.5107	60.0003	Time	
41	1	1-Vial 211	5885.83	60.0286	61.5001	Time	
42	1	1-Vial 212	5955.83	61.5111	63.0001	Time	
43	1	1-Vial 213	5960.83	63.0099	64.5001	Time	
44	1	1-Vial 214	5956.67	64.5109	66.0001	Time	
45	1	1-Vial 215	5442.50	66.0101	67.3707	Time	

=====

Appendix 6

Preparation of gels and buffers of SDS-PAGE

The gels, buffers and solutions were prepared as the follow:

1. Acrylamide/Bis-Acrylamide 40% (w/v) 37.5:1 purchased from Bio-Rad, USA.
2. Tetramethylethylenediamine (TEMED).
3. Sodium dodecyl sulphate (SDS) 10% was prepared by dissolving 1 g in 10 mL.
4. Ammonium persulphate (APS) 10%, 1 g were dissolved in 10 ml deionized water.
5. Stacking gel buffer 0.5M Tris-HCl pH 6.8, prepared by dissolving 6.1 g Tris-HCL in 80 mL deionized water to adjust the pH then completed to 100 mL in volumetric flask.
6. Separating gel buffer 1.5M Tris-HCl pH 8.8, prepared by dissolving 18.2 g Tris-HCL in 80 mL deionized water to adjust the pH then completed to 100 mL in volumetric flask.
7. Tank buffer consist of 0.025M Tris, 0.192M glycine and 0.1 % SDS, pH 8.3.
8. Fixing solution consisted of 50% methanol, 10% acetic acid (AcOH) and 40% dH₂O.
9. Staining solution, 0.025 g Commasie Blue (G-250) was dissolved in 100 ml of 10% acetic acid.
10. Destaining solution 10% AcOH.
11. Sample buffer consist of 12% SDS, 6% β -Mercaptoethano (AMRSCO, USA) 30% glycerol, 0.05% Commasie Blue (G-250) and 0.15M Tris-HCL pH 7.
12. Sample preparation: amount of 30 μ l of honey sample/fraction was mixed with 10 μ l of sample buffer and kept at 100 °C for 3 min.

13. Gel preparation and running the samples: the separation gel (16.5%) was poured in gel cassette, after getting solidified within 3 min then the stacking gel was poured and the combs fixed at the top. After the gel was solidified the combs removed, the gel cassette was transferred to the tank and pre-run for 10 min at 30 volts then 20 μ l of each sample was loaded to the wells, the second or third wells were loaded with the protein marker. Initial run was at 30 volts for 30 min to let the samples reached the separation gel, the power increased to 120 volts for 120 min or till the samples reached to the end of the gel. The gel then transferred to the fixing solution and kept for 30 min, and then the gel transferred to the staining solution and kept for 120 min. After staining the gel was destained several times till the bands the appeared.

LIST OF PUBLICATIONS

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