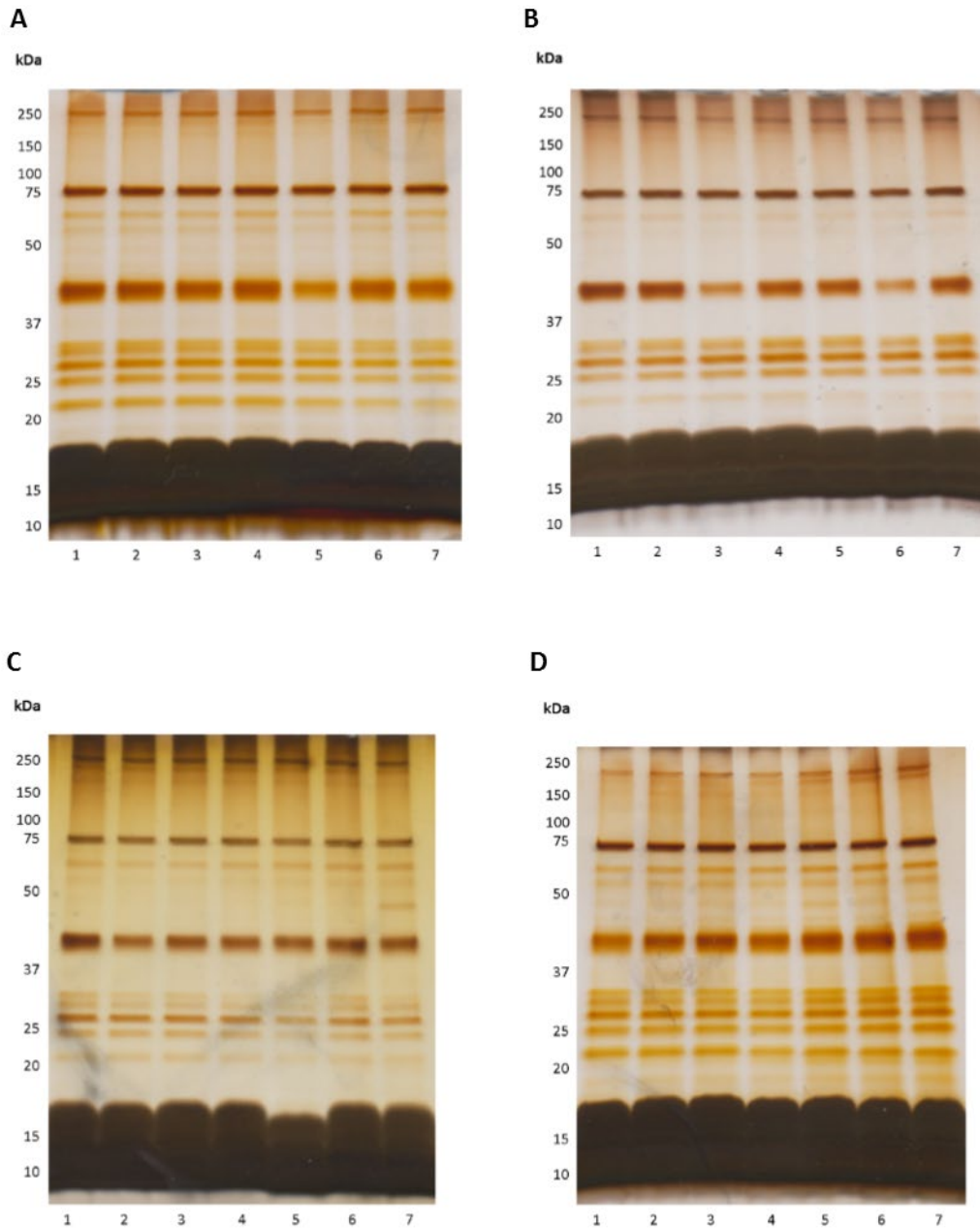


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2 **Supplementary Figure 1.** Representative chromatograms of JJAV samples containing (A) 100 µg/mL, (B) 50 µg/mL, (C) 25 µg/mL, and (D) 10
 3 µg/mL of venom protein. Samples were analysed using UPLC-UV coupled with Waters Acquity BEH C18 column and Photo Diode Array (PDA)
 4 detector. Peaks corresponding to the Myr p 1, Myr p 2, and Myr p 3 allergenic peptides are shown in panel (A).

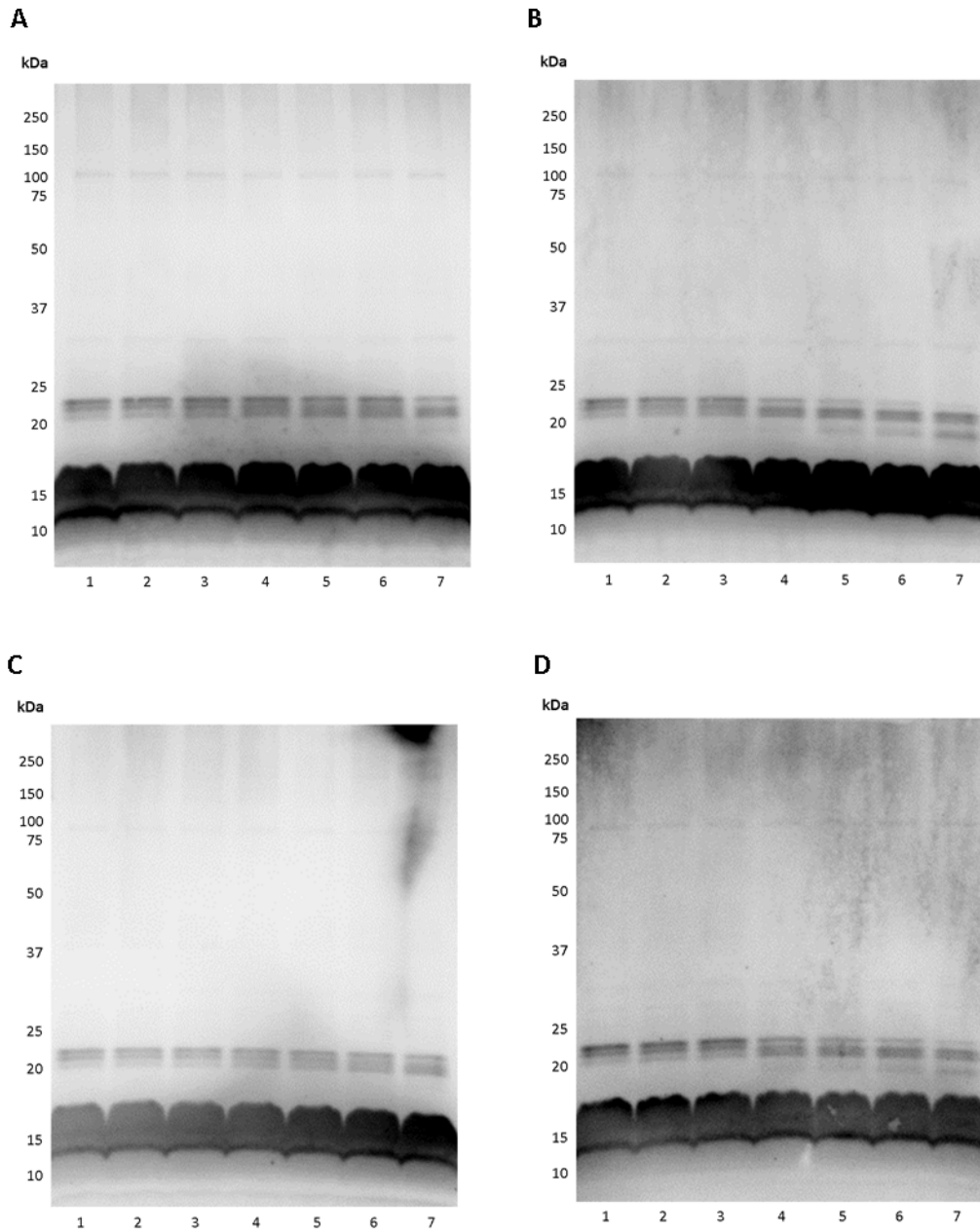
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2 **Supplementary Figure 2.** SDS-PAGE analysis of venom components in JJAV (25 µg/mL)
 3 stored at 4°C (A) without Advax and (C) with Advax (10 mg/mL), or at 25°C (B) without
 4 Advax and (D) with Advax (10 mg/mL) for up to 7 days. Lanes: 1. Baseline, 2. After 2 hours,
 5 3. After 6 hours, 4. After 1 day, 5. After 2 days, 6. After 3 days, and 7. After 7 days of storage.

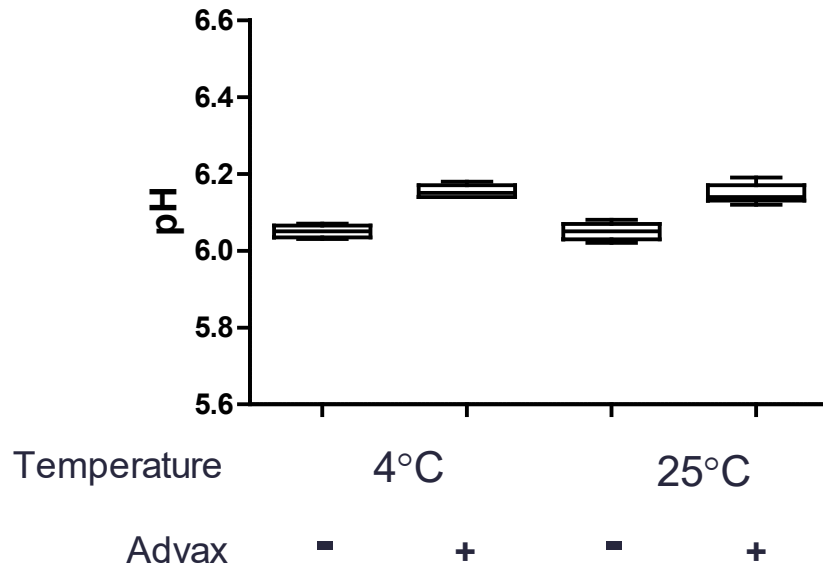
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2 **Supplementary Figure 3.** SDS-PAGE Immunoblot analysis of IgE binding venom
 3 components in JJAV (25 µg/mL) stored at 4°C (A) without Advax and (C) with Advax (10
 4 mg/mL), or 25°C (B) without Advax and (D) with Advax (10 mg/mL) for up to 7 days. Lanes:
 5 1. Baseline, 2. After 2 hours, 3. After 6 hours, 4. After 1 day, 5. After 2 days, 6. After 3 days,
 6 and 7. After 7 days of storage.

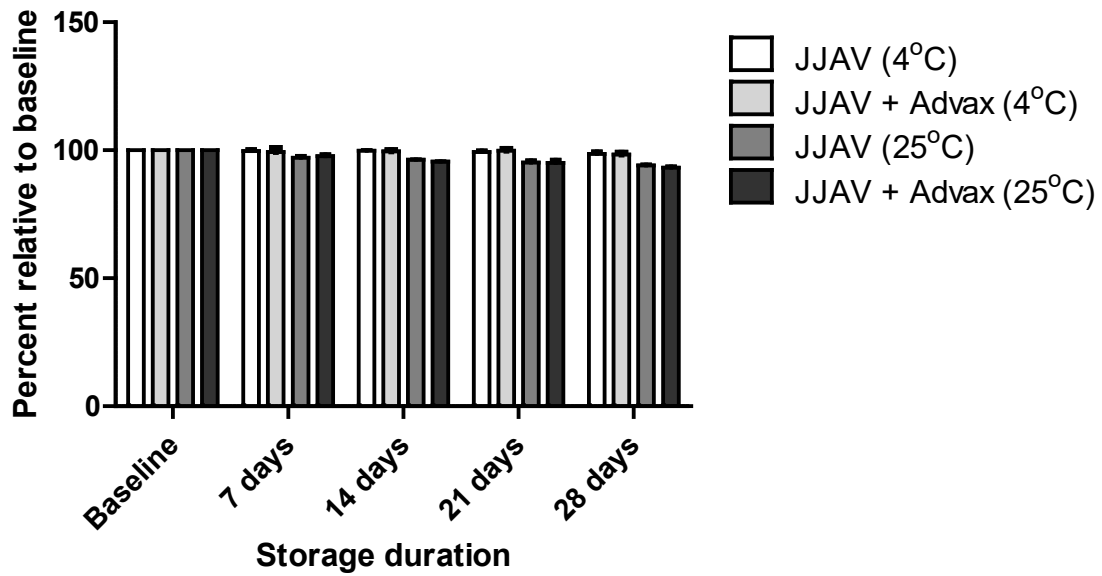
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2 **Supplementary Figure 4.** Effects of Advax (10 mg/mL) and storage temperature (4°C or
 3 25°C) on the buffering capacity of phosphate buffered saline in JJAV diluent used in the
 4 formulation of JJAV (25 µg/mL) with or without Advax. Analysis of pH was taken from each
 5 samples at defined time points for a period of 28 days and presented as mean and SD.

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2 **Supplementary Figure 5.** Effects of Advax (10 mg/mL) and storage temperature (4°C or
 3 25°C) on benzyl alcohol concentration in JJAV diluent used in the formulation of JJAV (25
 4 µg/mL) with or without Advax. Benzyl alcohol concentration in the samples was quantified
 5 against an analytical standard using UPLC-UV. Analysis was calculated as a percentage
 6 relative to baseline samples. Quadruple analysis was performed on each sample and presented
 7 as mean and SD.

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Supplementary Table 1. Allergenic potency of various JJAV concentrations with or without Advax at baseline as determined by JJAV-specific IgE ELISA Inhibition assay

| JJAV Concentration | Storage Condition | Advax adjuvant | Allergenic Potency as 50% Inhibition (Mean \pm SD; nanogram) | P value |
|--------------------|-------------------|----------------|--|---------|
| 25 μ g/mL | 4°C | – | 0.349 \pm 0.072 | 0.60 |
| | | + | 0.385 \pm 0.081 | |
| | 25°C | – | 0.501 \pm 0.061 | |
| | | + | 0.424 \pm 0.138 | |
| 10 μ g/mL | 4°C | – | 0.301 \pm 0.002 | 0.99 |
| | | + | 0.304 \pm 0.230 | |
| | 25°C | – | 0.276 \pm 0.065 | |
| | | + | 0.335 \pm 0.204 | |
| 1 μ g/mL | 4°C | – | 0.648 \pm 0.316 | 0.35 |
| | | + | 0.441 \pm 0.113 | |
| | 25°C | – | 0.480 \pm 0.230 | |
| | | + | 0.291 \pm 0.114 | |
| 0.1 μ g/mL | 4°C | – | 0.297 \pm 0.007 | 0.06 |
| | | + | 0.628 \pm 0.118 | |
| | 25°C | – | 0.224 \pm 0.001 | |
| | | + | 0.461 \pm 0.116 | |

P values were calculated using t-test; p < 0.05 was considered significant

16 **Supplementary Table 2.** Concentration of Myr p allergens at baseline in formulations
 17 containing JJAV (25 µg/mL) with or without Advax as determined by UPLC-UV
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| Storage Condition | Advax adjuvant | Allergen | AUC at Baseline (Mean ± SD) |
|-------------------|----------------|----------|-----------------------------|
| 4°C | – | Myr p 1 | 3039.22 ± 84.95 |
| | | Myr p 2 | 9421.88 ± 322.82 |
| | | Myr p 3 | 378.76 ± 23.26 |
| | + | Myr p 1 | 3151.46 ± 161.53 |
| | | Myr p 2 | 9730 ± 541.86 |
| | | Myr p 3 | 392 ± 43.71 |
| 25°C | – | Myr p 1 | 3249.23 ± 102.43 |
| | | Myr p 2 | 9757.38 ± 412.2 |
| | | Myr p 3 | 415.93 ± 11.21 |
| | + | Myr p 1 | 3179.17 ± 131.13 |
| | | Myr p 2 | 9673.43 ± 257.06 |
| | | Myr p 3 | 412.68 ± 33.95 |

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Supplementary Table 3. Endotoxin level in multiple batches of JJA VIT, JJAV diluents and Advax adjuvant used in the study

| Sample | Number of batches | Mean \pm SD (EU/mL) |
|----------------|--------------------------|---|
| JJA VIT | 5 | 0.028 \pm 0.001 |
| JJAV diluent | 5 | 0.022 \pm 0.001 |
| Advax adjuvant | 3 | 1.534 \pm 0.020 |

68 **Supplementary Table 4.** Antimicrobial efficacy of benzyl alcohol in JJAV diluent used in the formulation of JJAV (25 µg/mL) with or without
 69 Advax (10 mg/mL)
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| Sampling time | Sample | Observed Log reduction (Mean of 5 plates) | | | Acceptance criterion |
|---------------|-------------------|---|--------------------------------------|--------------------------------------|---|
| | | <i>E. coli</i> ATCC 25922 | <i>S. aureus</i> ATCC 25923 | <i>C. albicans</i> ATCC 10231 | |
| Baseline | 0.9% NaCl Control | 1 ± 1 x 10 ⁶ CFU/plate | 4 ± 1 x 10 ⁵ CFU/plate | 3 ± 3 x 10 ⁵ CFU/plate | 1 x 10 ⁵ to 1 x 10 ⁶ CFU/plate on initial count |
| Day 7 | JJAV | 6 Log reduction on both sample types | 5 Log reduction | 5 Log reduction on both sample types | ≥ 1 log reduction from the initial count |
| | JJAV + Advax | | 3 Log reduction | | |
| Day 14 | JJAV | 6 Log reduction on both sample types | 5 Log reduction on both sample types | 5 Log reduction on both sample types | ≥ 3 log reduction from the initial count |
| | JJAV + Advax | | | | |
| Day 28 | JJAV | 6 Log reduction on both sample types | 5 Log reduction on both sample types | 5 Log reduction on both sample types | No increase from the 14 day count |
| | JJAV + Advax | | | | |

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