**Table** **S1** Propolis extracts used in screening for anti-*Acanthamoeba* activity

|  |  |
| --- | --- |
| **Propolis from City** | **Coordinate** |
| No. 1 Tabriz city | 38.0962° N, 46.2738° E |
| No. 2 South Khorasam province | 32.5176° N, 59.1042° E |
| No. 3 Pranshahr city | 36.6950° N, 45.1446°E |
| No. 4 Mashhad city | 36.2605° N, 59.6168° E |
| No. 5 Sarab city | 37.9429° N, 47.5384° E |
| No. 6 Sardasht county | 36.1584° N, 45.4760° E |
| No. 7 Boroujen city | 31.9798° N, 51.2946° E |
| No. 8 Neyshabor city | 36.2141° N, 58.7961° E |
| No. 9 Tehran city | 35.6892° N, 51.3890° E |
| No. 10 Kermanshah city | 34.3277° N, 47.0778° E |

**Table S2** Eye drops composition

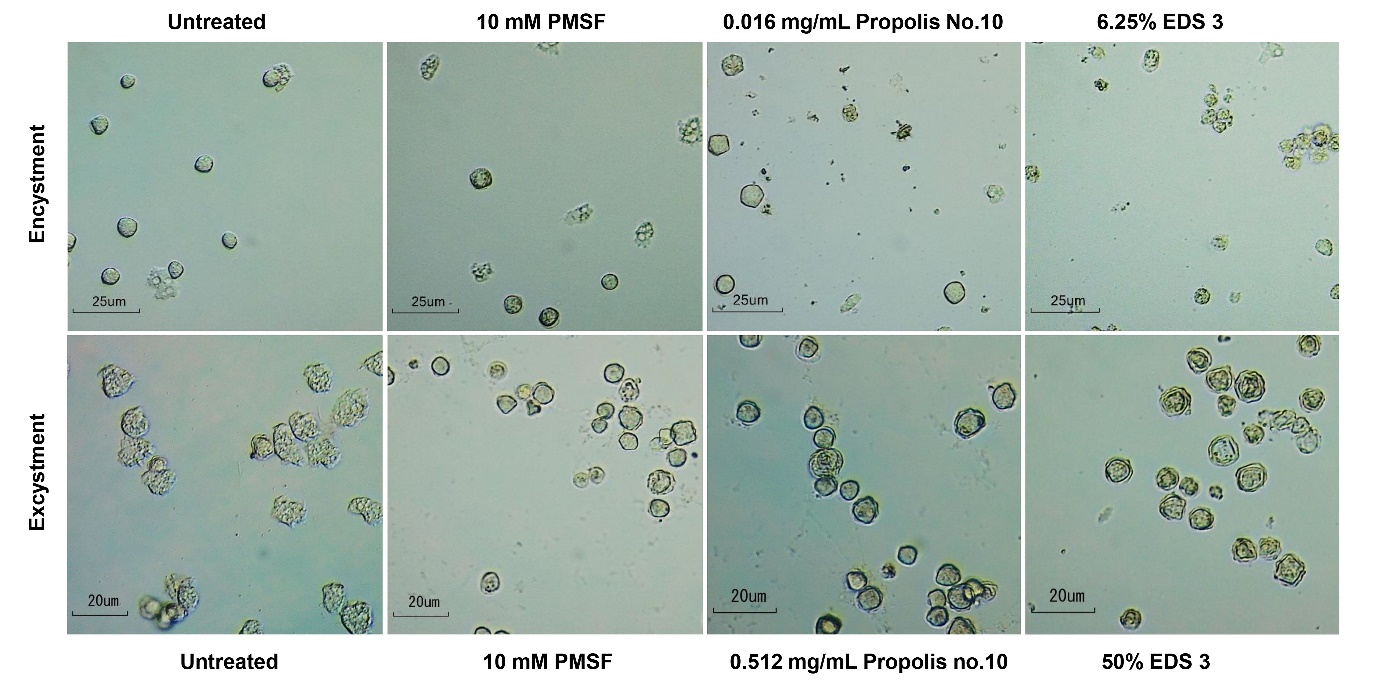
|  |  |
| --- | --- |
| **Eye drops solution (ESD)** | **Composition** |
| EDS-1 | Chloramphenicol 5 mg/mL |
| EDS-2 | Neomycin sulfate 2 mg/mL  Gramicidin 0.025 mg/mL  Polymyxin B 5000 units |
| EDS-3 | Antazoline HCI 0.5 mg/mL  Tetrahydrozoline HCl 0.4 mg/mL  Benzalkonium chloride 0.15 mg/mL |
| EDS-4 | Levofloxacin 5 mg/mL |

**Table S3** Screening of anti-*Acanthamoeba* activity from plant and Propolis extracts

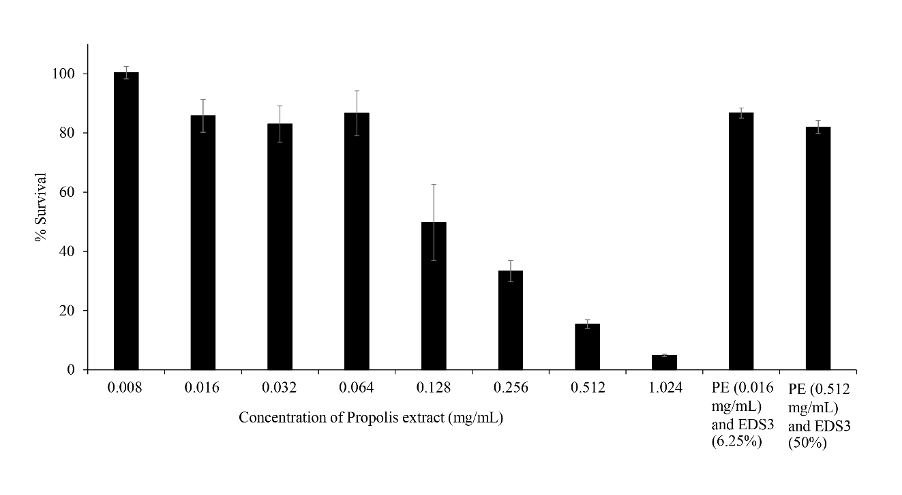
|  |  |  |
| --- | --- | --- |
| **Plant name/Propolis** | **Percentage of viable cells at 1 mg/mL** | |
| **Trophozoites** | **Cysts** |
| No. 1 Tabriz city | 0 | 38.46 ± 7.69 |
| No. 2 South Khorasam province | 32.85 ± 8.92 | 79.48 ± 4.44 |
| No. 3 Pranshahr city | 0 | 74.35 ± 4.44 |
| No. 4 Mashhad city | 11.76 ± 5.09 | 87.17 ± 4.44 |
| No. 5 Sarab city | 0 | 82.05 ± 4.44 |
| No. 6 Sardasht county | 70.58 ± 8.82 | 46.15 ± 5.38 |
| No. 7 Boroujen city | 44.28 ± 2.47 | 51.28 ± 4.44 |
| No. 8 Neyshabor city | 79.41 ± 0 | 64.10 ± 8.88 |
| No. 9 Tehran city | 70.58 ± 8.82 | 79.48 ± 9.35 |
| No. 10 Kermanshah city | 0 | 5.12 ± 8.88 |
| **Eye drop solution** | **Percentage of viable cells at 100%** | |
| **Trophozoites** | **Cysts** |
| EDS 1 | 0 | 23.08 ± 6.88 |
| EDS 2 | 2.86 ± 4.42 | 13.08 ± 13.71 |
| EDS 3 | 8.57 ± 0 | 7.69 ± 0 |
| EDS 4 | 25.71 ± 0 | 43.59 ± 10.51 |

**Table S4** Minimal inhibitory concentration (MIC) of active extract, eye drops and chlorhexidine against *Acanthamoeba triangularis* WU19001 trophozoites and cysts.

|  |  |  |
| --- | --- | --- |
| **Anti-*Acanthamoeba* agent** | **MIC (mg/mL)** | |
| **Trophozoites** | **Cysts** |
| No. 1 Propolis-Tabriz city | 0.256 | > 1 |
| No. 3 Propolis-Pranshahr city | 0.128 | > 1 |
| No. 5 Propolis-Sarab city | 0.128 | > 1 |
| No. 10 Propolis-Kermanshah city | 0.256 | 1 |
| EDS-1   * Chloramphenicol | 100%  5 | > 100%  > 5 |
| EDS-2   * Neomycin Sulfate * Gramicidin * Polymyxin B | 100%  2  0.025  5000 units | > 100%  > 2  > 0.025  > 5000 units |
| EDS-3   * Antazoline HCI 0.5 * Tetrahydrozoline HCl 0.4 * Benzalkonium Chloride 0.15 | 100%  0.5  0.4  0.15 | 100%  0.5  0.4  0.15 |
| EDS-4  - Levofloxacin | > 100  > 5 | > 100  > 5 |
| Chlorhexidine | 0.008 | 0.032 |



**Fig. S1** Encystment and excystment of *A. triangularis* in PYG and Neff’s medium with and without propolis extract No. 10 and eye drops EDS 3. Magnification = 40x



**Fig. S2** Toxicity of various concentrations of Propolis extract and combination sets.