**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/mLGramicidin 0.025 mg/mLPolymyxin B 5000 units |
| EDS-3  | Antazoline HCI 0.5 mg/mLTetrahydrozoline HCl 0.4 mg/mLBenzalkonium 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% 20.0255000 units | > 100% > 2> 0.025> 5000 units |
| EDS-3* Antazoline HCI 0.5
* Tetrahydrozoline HCl 0.4
* Benzalkonium Chloride 0.15
 | 100% 0.50.40.15 | 100% 0.50.40.15 |
| 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.