**Table S1.** List of primers for real-time PCR.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene** | **Genbank Accession No.** | **Forward (F)** | **Reverse (R)** | ***Acanthamoeba* spp.** | **References** |
| ATG16 | FJ906697 | 5’-AGCTTGACTTCCATCACGCTGA-3’ | 5’-TGTTTGAGGTTGGCCCGAA-3’ | *A. castellanii* | (Song *et al.*, 2012) |
| ATG3 | - | 5’-GCGCACGTACGATATCTCCATC-3’ | 5’-ATGAACACTTGGTTCGGCGTC-3’ | *A. castellanii* | (Moon *et al.*, 2011) |
| ATG8b | KC524507.1 | 5’-CCGAGTTCCTGTGATCGTTGA-3’ | 5’-AGCTGTGTGACGGCAATATCG-3’ | *A. castellanii* | (Moon *et al.*, 2013) |
| 18S rRNA | - | 5′-TCCAATTTTCTGCCACCGAA-3′ | 5′-ATCATTACCCTAGTCCTCGCGC-3′ | *A. castellanii* | (Song *et al.*, 2012), (Moon *et al.*, 2008) |

**Table S2.** Minimal inhibitory concentration of *Cassia angustifolia* extract against *Acanthamoeba triangularis*.

|  |  |
| --- | --- |
| ***Cassia angustifolia* extract** | **MIC (µg/mL)** |
| **24h** | **48h** | **72h** |
| **Trophozoite** | 1024 | 2048 | 2048 |
| **Cyst** | >2048 | >2048 | >2048 |

**Table S3.** *Acanthamoeba triangularis* DNA sequence by Sanger sequencing.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Target gene** | **Primer** | **DNA sequence** | **Product length (bp).** | **% Identity**a |
| **ATG16** | F | 5’-TCCGGGCCCTCTCCGGCTTACGCCCGCCACCTCACAGAACCCTTCAAAGCTAACGAGAGTCACGCCACACAGATGGAAGCTCGATCCTGTCCAACTCAAGAGACAGCAGCCTCAAGATGCTTGACATTCGGGCCAACCTCAAACAAA-3’ | 147 | 97.10 |
|  | R | 5’-AAAAGTTGAGCTGCTGTCTCTTGAGTTGGACAGGATCGAGCTTCCATCTGTGTGGCGTGACTCTCGTTAGCTTTGAAGGGTCTGTGAGGTGGCGGGCGTAGCGGAGAGGGGTACCCGGGCTCAGCGTGATGGAAGTCAAG-3’ | 140 | -b |
| **ATG3** | F | 5’-AAAATACAGAACGCCCAAGGTGTGGCTGTTTGGCTACGACGAGGTACACCCTGCTTTGACCCCGTACCGCTCGGGTGGCCACCCGCAGACAAGGCAAAGCAACTGATTCTTCTTTGTGCCCTCTATTTCTATCCGCGGCGTGTAGAATGGCAACGGCCTGACGCCGAACCAAGTGTTCATA-3’ | 181 | 100.00 |
|  | R | 5’-NTCTACGCCGCGGATAGAATAGAGGGCACAAAGAAGAATCAGTTGCTTTGCCTTGTCTGCGGGTGGCCACCCGAGCGGTACGGGGTCAAAGCAGGGTGTACCTCGTCGTAGCCAAACAGCCACACCTTGGGCGTCTGGTAGTACTTGTCGTAGGTGATGGAGATATCGTACGTGCGCA-3’ | 178 | 100.00 |
| **ATG8b** | F | 5’-CAGGGACAGCTCTTCCGACTTTCCAGAGGAGTACGCCTTGCGCCTTGCACCTATCCTCTCTATCCATGCTGCTGAAAGTTGCTCTTCGGTTCTCGGCTCCTCGTGGATATGCCCTCTCCACCTATAGGC-3’ | 129 | -b |
|  | R | 5’-CCCCCNACTGCGGAAGGAACTTTCTTCAGCATCTGGATATGGGATCTACGTGCAAGTGCAACGCATACATTTTCTCTTAAATATCTCGAATAAGATCTCTCCCCTCGTGCTCTTCTCCAATATCACGAGAACTCGGAC-3’ | 138 | -b |
| **18S rRNA** | F | 5’-AATGGAATGGAATAGGACCTGTCCTCCTATTTTCAGTTGGTTTTGGCAGCGCGAGGACTAGGGTAATGATA-3’ | 71 | 98.48 |
|  | R | 5’-TGAAATTAGGAAGGAACGGGTCCTATTCCATTATCCCATGCTAATGTATTCCGGTGGCAGAAAATTGGAAAATAGGAC-3’ | 78 | 92.75 |

a The DNA sequences were blasted against *A. castellanii* strain in NCBI database.

b No significant similarity found by NCBI-DNA blast (*A. castellanii* ATCC30011).