Inhibitory and anti-adherent effects of piper betle L. leaf extract against acanthamoeba triangularis in co-infection with staphylococcus aureus and pseudomonas aeruginosa: A sustainable one-health approach

ABSTRACT

Background and Aim: Keratitis is a serious ocular infection often caused by pathogenic microorganisms such as Acanthamoeba spp. Among other harmful microbes, Acanthamoeba keratitis presents a particular challenge due to its resistance to conventional antimicrobial agents. Piper betle Linn., commonly known as betel leaf, has been traditionally used for its medicinal properties. This study aimed to assess the potential of the leaf ethanol extract of P. betle Linn. in the treatment of Acanthamoeba triangularis in monoculture and co-culture with two prevalent pathogenic bacteria, Staphylococcus aureus and Pseudomonas aeruginosa, associated with keratitis. Materials and Methods: Minimum inhibitory concentrations (MICs) of A. triangularis, S. aureus, and P. aeruginosa extracts in monoculture and coinfected conditions were examined. In addition, this study explored the potential of the extract in preventing Acanthamoeba adherence in both monoculture and co-culture environments. Scanning electron microscopy (SEM) analysis confirmed the impact of the extract on Acanthamoeba cell membranes, including acanthopodia. Furthermore, a timekill kinetic assay was used to validate the amoebicidal activity of the extract against A. triangularis and the tested bacteria. Results: MICs for trophozoites, cysts, P. aeruginosa, and S. aureus in the monoculture were 0.25, 0.25, 0.51, and 0.128mg/mL, respectively, whereas the MICs for Acanthamoeba coinfected with bacteria were higher than those in the monoculture. This extract inhibited the growth of A. triangularis trophozoites and cysts for up to 72 h. Moreover, P. betle extract effectively prevented the adherence of Acanthamoeba to contact lenses under monoculture conditions. SEM analysis confirmed that P. betle extract affects the cell membrane of Acanthamoeba, including Acanthopodia. In addition, the time-kill kinetic assay confirmed that the extract contained amoebicidal activity against A. triangularis, including the tested bacteria. Notably, S. aureus was more susceptible than A. triangularis and P. aeruginosa to P. betle extract treatment. Unexpectedly, our study revealed that S. aureus negatively affected A. triangularis in the co-culture after 3 days of incubation, whereas P. aeruginosa facilitated the growth of A. triangularis in the presence of the extract. Conclusion: This study provides compelling evidence of the anti-adhesive and anti-Acanthamoeba properties of P. betle leaf extract against A. triangularis under monoculture and co-culture conditions. The observed impact on Acanthamoeba cell membranes, coupled with the time-kill kinetic assay results, underscores the potential of P.

betle leaf extract as a promising agent for combating Acanthamoeba-related infections in humans and animals.