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Antimicrobial peptides in web silk of *Nephilengys malabarensis* (hermit spider) are secreted by the aggregate gland

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Increasing prevalence of infections caused by multidrug-resistant bacteria has become a global health issue due to the shortage of novel classes of antibiotics. The current study was conducted to assess the antibacterial activity of web silk secreted by *Nephilengys malabarensis*, the hermit spider. Natural silk, spun silk, and silk gland secretions were screened for antibacterial activity against gram positive*Staphylococcus aureus*and *Bacillus subtilis,* and gram negative*Escherichia coli*and*Pseudomonas aeruginosa* bacterial species. Natural silk was obtained directly from the web and spun silk was taken through artificial forced spinning. All silk samples and homogenized gland secretions were tested under the agar disc method and resazurin assay for antibacterial activity. Tetracycline was used as the positive control and saline as the negative control. All assays were repeated with a minimum of N=9. Data were analyzed with statistically significant values defined as $p<0.05$ based on one-way analysis of variance (ANOVA) using SPSS statistics 20. The study revealed a significant inhibition of bacterial growth by the aggregate gland ($f= 18.46$, $p= 0.000$). The mean difference between positive control (Tetracycline) and the aggregate gland secretion was significantly similar at the 95% confidence level ($p= 1.000$). The extractions were resolved via SDS-PAGE and an agar overlay assayconducted. Further antibacterial activity was assayed after digestion with proteinase K. The inhibition zones present on the agar overlay plate and loss of activity after proteinase K treatment indicate that the active agent is a non-diffusible protein. Therefore, the results obtained strongly suggest that the peptides derived from *Nephilengys malabarensis* silk glands have antibiotic properties, which can be further investigated as antibacterial drug leads.

Keywords: multidrug-resistant bacteria, antibacterial activity, silk glands, agar overlay assay

Acknowledgment: University of Colombo is acknowledged for financial support.

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