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Spider silk as a potential antibiotic substitute

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ABSTRACT: Studies were undertaken on the antibacterial activity of spider silk against bacterial strains, using egg case silk of *Parawixia dehaani* (Doleschall, 1859) and web silk of *Pholcus phalangioides* (Fuesslin, 1775). Both silk types inhibited gram negative bacteria more than gram positive bacteria. The egg case silk of *P. dehaani* showed more antimicrobial activity than the web silk of *P. phalangioides*. The egg case silk brought about 26.05 per cent inhibition against *E. coli*, compared to a 22.7 per cent inhibition for *B. subtilis*. A linear association was found between the volume of silk extract and the percentage of inhibition. The percentage of inhibition against *E. coli* increased from 5.13 to 26.05 per cent as the volume rose from 20 to 100 µl. in *P. dehaani.* © 2022 Association for Advancement of Entomology

KEYWORDS: *Parawixia dehaani*, *Pholcus phalangioides*, antibacterial, web silk, egg case silk

Spiders have long piqued people's interest due to their unique ability to weave webs with geometrical precision and artistry (Soler and Zaera, 2016; Vollrath and Krink, 2020). Spiders use their silk for a variety of purposes, including as a lifeline that allows them to safely flee from predators, as a shelter, as a snare, and also to guard their eggs (Römer and Scheibel, 2008; Iqbal *et al.,* 2019). Their capacity to produce multiple types of silk from distinct glands enables them to rely on silk as a means for survival. The cynosure here is the egg case silk and web silk produced by aciniform, tubuliform and ampullate (major and minor) glands of spiders. Web silk, including drag line, that has tensile strength greater than high tensile engineering steel (Sebastian and Peter, 2009; Kono *et al.*, 2021) and egg case silk, which has its own methods of enduring environmental risks such as infections from outside sources (Vierra *et al.*, 2011), are both valuable biomimetic prospects, with some of them already being actively utilized in research.

Spider silk is being studied for its antibacterial effects in addition to its use as a mechanical biomimetic. The idea of examining spider silk for antibacterial potential appears to have been sparked by historical reports of wound healing using spider silk. Amaley *et al*. (2014) using drag line silk of *Nephila pilipes* (Fabricius, 1793) (Family: Araneidae) against different bacteria including *Escherichia coli*, and Keiser *et al*. (2015) employing social spider *Stegodyphus dumicola* Pocock 1898 (Family: Eresidae) against *Bacillus thuringiensis* illustrated the antibacterial efficacy of spider silk. While several studies revealed that spider silk has antibacterial properties (Sawarkar and Sawarkar, 2017; Phartale *et al*., 2019; Deshmukh and Pansare, 2019; Sangavi *et al*., 2020) others dismissed it as a hoax or perhaps a methodological flaw (Fruergaard *et al*., 2021).

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The focus of this research is to evaluate the antibacterial properties of two types of spider silk collected from two different spider species.

Web silk of *Pholcus phalangioides* (Fuesslin, 1775) (Family: Pholcidae) and egg case silk of *Parawixia dehaani* (Doleschall, 1859) (Family: Araneidae) were collected to evaluate the antimicrobial property. Spiders were collected from Kandakkadavu wetlands (Ernakulam district, Kerala, India). Spiders were reared separately in large sterile plastic bottles of 200 ml size (100 mm tall and 56 mm in diameter). Mosquitoes were offered to spiders on daily basis. Web silk made by *P. phalangioides* inside the bottle was collected and preserved in sterile conditions. *P. dehaani* laid eggs in the bottle and the silk from its egg case was collected after the emergence of spiderlings and preserved. Gram negative *Escherichia coli* (MTCC NO 2414) and gram positive *Bacillus subtilis* (MTCC NO 443) were the bacterial strains chosen for the test. Antibacterial assay was carried out using the microtiter plate method with an ELISA reader (Thermo Scientific, USA) under aseptic conditions.

Preparation of silk extract and microbial culture: One gram of spider silk was weighed and hydrolyzed with 10 ml of acetone for one week at 30°C to make the silk sample. The hydrolyzed cobweb was centrifuged for 30 minutes at 4000 rpm. To ensure decontamination, the supernatant was then passed through a filter with a pore size of 0.4 µm and refrigerated. For the microbial culture, a single pure colony of *E. coli* and *B. subtilis* was loaded into a 10 ml nutrient broth (HiMedia) tube, sealed, and incubated at 37°C overnight (incubator used REMI). Then, using McFarland standards as a reference, the turbidity of suspensions was estimated and adjusted.

Preparation of the microtiter plates: In the current study, microtiter plates were used for the antimicrobial assay. A varied volume of silk sample (20 µl, 40 µl, 60 µl, 80 µl and 100 µl), 100 µl of nutrient broth and microbial suspension were added to each well of the microtiter plate. Control dilutions of test material were also kept. As organism control, a column was prepared with all solutions excluding the silk sample. To keep the culture from being dried, the plate was wrapped loosely in cling film. After a 24 hour incubation period at 37°C, an OD reading was taken (OD_{600}) using spectrophotometer (Thermo Scientific, USA). The experiment was repeated twice and the mean values were tabulated (Table 1 and 2).

Optical density was calculated by subtracting the control OD from the sample OD and the percentage of inhibition was calculated from the following equation,

Percentage of inhibition =
[(Control –Test) / Control] ×100

where, OD of extract is the difference between test OD and final test OD

The egg case silk of *P. dehaani* showed more antimicrobial activity than the web silk of *P. phalangioides*. Both silk samples showed more inhibition against the gram negative bacteria, *E. coli*. As the volume of extract was raised from 20 to 100 µl, a progressive increase in the percentage of inhibition was observed. However, in both silk samples, a significant percentage was detected only at 100 µl (Table 1, 2).

Only a few studies have been conducted on the antibacterial activity of egg case silk. Wright (2011) observed antibacterial properties in the egg case silk of the spider *Pityohyphante sphrygianus* (C.L. Koch, 1836) (Family: Linyphiidae) against *E. coli* and *B. subtilis*. The finding of the present investigation is consistent with this result. The presence of water-soluble, polar coating peptides isolated from egg sac silk, which have been theorized to be antibacterial, could be one rationale (Vierra *et al*., 2011).

In present investigation, antibacterial activity in the web silk of *P. phalangioides* was observed in both gram positive and gram negative bacteria, with gram negative bacteria being more prone to inhibition. Tahir *et al*. (2017) employed silk from *Cyclosa confraga* (Thorell, 1892) to reach the same conclusion. In 2018, Tahir *et al*. used silk of

P. phalangioides in a comparable study and established the vulnerability of gram negative bacteria. Gram negative bacterial strains were again found to be more susceptible than gram positive strains in an experiment where the antibacterial capabilities of spider silk against multidrug resistant bacteria were analyzed (Haq *et al*., 2019). The heightened sensitivity of gram negative bacteria can be explained by *E. coli*'s limited bacterial adhesion to the silk surface (Sharma, 2014). The thin peptidoglycan coating in gram negative bacteria could also be a possible reason. Gram positive bacteria may be more resistant to spider silk because they have a thick peptidoglycan coating and teichoic acids in their cell walls (Brown *et al*., 2013). The presence of compounds such as Gamma-aminobutyric acid (GABA) in the silk, which serves as a defense mechanism against natural enemies rather than as a silk byproduct, contributes to the bacteriostatic quality of the silk (Zhang *et al*., 2012). However, there are certain other studies thatcontradict our results. Silk inhibition was found to be more effective against gram positive bacteria than gram negative bacteria (Wright, 2011; Mirghani *et al*., 2012; Roozbahani *et al*., 2014; Al Kalifawi and Kadem, 2017). The linear relationship between the concentration of silk sample and the inhibition percentage corroborates with the study conducted by Tahir *et al*. (2018, 2019) in *Eriovixia excelsa* (Simon, 1889) where they used increasing concentrations of silk in sodium hydroxide (NaOH) solvent solution. Understanding the capabilities of spider silk has opened the door to a larger range of uses, including therapeutic ones, which are perhaps the most exciting prospects and call for further research.

Table 1 **Antimicrobial activity of egg sac silk of *Parawixia dehaani* against *B. subtilis* and *E. coli***

Bacteria	OD/ Inhibition	Concentration μl				
		20	40	60	80	100
B. subtilis	Final test OD	0.704	0.669	0.628	0.599	0.563
	Inhibition (%)	3.43	8.23	13.85	17.83	22.7
E. coli	Final test OD	0.998	0.948	0.876	0.844	0.778
	Inhibition (%)	5.13	9.89	16.73	19.77	26.05

Table 2 **Antimicrobial activity of web silk of *Pholcus phalangioides* against *B. subtilis* and *E. coli***

Bacteria	OD/ Inhibition	Concentration μl				
		20	40	60	80	100
B. subtilis	Final test OD	0.712	0.690	0.673	0.660	0.641
	Inhibition (%)	2.33	5.35	7.68	9.47	12.07
E. coli	Final test OD	1.008	0.990	0.972	0.922	0.873
	Inhibition (%)	4.18	5.89	7.60	12.36	17.02

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REFERENCES

Al-Kalifawi E.J. and Kadem Y.J. (2017) The antimicrobial activity of Al-Ankabut's home (spider's web) extract. Mesopotemia environmental journal Special Issue C: 54-63.

Amaley A., Gawali A.A. and Akarte S.R. (2014) Antibacterial nature of dragline silk of *Nephilapilipes* (Fabricius, 1793). Indian Journal of Arachnology 3(1): 8-11.

Brown S., Santa Maria J.P. and Walker S. (2013) Wall Teichoic Acids of Gram Positive Bacteria. Annual Review of Microbiology 67(1): 313–336. doi:10.1146/annurev-micro-092412-155620.

Deshmukh U.S. and Pansare A.S. (2019) Antimicrobial activity of web of spider, *Stegodyphussara senorum* on *E. coli* and *S. aureus*. Bioscience Biotechnology Research Communications 12: 787-788.

Fruergaard S., Lund M.B., Schramm A., Vosegaard T. and Bilde T. (2021) The myth of spider silk. iScience 24: 103125. doi.org/10.1016/j.isci.2021.103125

Haq I.U., Qasim M., Rahim K., Ameen F., Dawoud T., Basit A., Munir S. and Alshehrei F. (2019) Efficacy of the spider web metabolites activity against multi drug resistance (MDR) bacteria. Applied Ecology and Environmental Research 17(5): 10899-10908.

Iqbal Z., Liaqat M., Shaukat A., Sabri M.U. and Akhtar M. (2019) Antimicrobial activity of extracts of spider web silk. World Journal of Pharmaceutical Research 8(11): 106-109. doi: 10.20959/wjpr201911-15657.

Keiser C.N., DeMarco A.E., Shearer T.A., Robertson J.A. and Pruitt J.N. (2015) Putative microbial defenses in a social spider: immune variation and antibacterial properties of colony silk. Journal of Arachnology 43: 394-399.

Kono N., Nakamura H., Mori M., Yoshida Y., Ohtoshi R., Malay A.D., Pedrazzoli Moran D.A., Tomita M., Numata K. and Arakawa K. (2021) Multi component nature underlies the extraordinary mechanical properties of spider dragline silk. Proceedings of the National Academy of Sciences of the United States of America 118(31): e2107065118. doi.org/10.1073/pnas.2107065118.

Mirghani M., Kabbashi N., Elfaki F. and Zulkifli M. (2012) Investigation of the spider web for antibacterial activity. Malaysian International Conference on Trends in Bioprocess Engineering, BT-201-1-BT-201-5. http://irep.iium.edu.my/26101/1/[BT-201]_Mohamed_Elwathiq.pdf.

Phartale N.N., Kadam T.A., Bhosale H. J., Karale M.A. and Garimelle G. (2019) Exploring the antimicrobial potential of *Pardosa brevivulva* silk. Journal of Basic and Applied Zoology 80 (1): 31-39. doi.org/10.1186/s41936-019-0102-6.

Römer L. and Scheibel T. (2008) The elaborate structure of spider silk: structure and function of a natural high-performance fiber. Prion 2(4): 154-161. doi:10.4161/pri.2.4.7490.

Roozbahani H., Asmar M., Ghaemi N. and Issazadeh K. (2014) Evaluation of antimicrobial activity of spider silk *Pholcus phalangioides* against two bacterial pathogens in food borne. International Journal of Biological and Biomedical Research 2(7): 2197-2199.

Sangavi D., Douglas J., Shyu H. and Nathan P.T. (2020) Antimicrobial activities of the silk produced by two species of spiders *Pardos*a s*umatrana* and *Stegodyphus sara sinorum.* Physical, Chemical and Biological Sciences: Emerging Trends and Milestones in 2020. Chapter 23. ISBN: 978-81-942052-9-6. 236-244 pp.

Sawarkar A.S. and Sawarkar S.B. (2017) Medicinal prospects for major ampullate silk of giant wood spider, *Nephila pilipes*. International Journal of Researches in Biosciences, Agriculture and Technology 5(2): 159-161.

Sebastian P.A. and Peter K.V. (2009) Spiders of India. University Press (India) Pvt. Ltd, Hyderabad. 614 pp.

Sharma S. (2014) Investigation of antimicrobial properties of spider silk. Master of Science Thesis, University of Akron.1-49 pp.

Soler A. and Zaera R. (2016) The secondary frame in spider orb webs: the detail that makes the difference. Scientific Reports 6: 31265. doi: 10.1038/srep31265.

Tahir H.M., Qamar S., Sattar A., Shaheen N. and Samiullah K. (2017) Evidence for the antimicrobial potential of silk of *Cyclosa confraga* (Thorell, 1892) (Araneae: Araneidae). Acta Zoologica Bulgarica 69(4): 593-595.

Tahir H.M., Sattar A., Qamar S., Mukhtar M.K., Liaqat I., Ali M.A., Zaheer A., Arshad N., Yaqoob R. and Naseem S. (2019) Anti-bacterial potential of silk recovered from *Eriovixia excelsa* (Simon, 1889) spider. Journal of Animal and Plant Sciences 29(2): 625-628.

Tahir H.M., Zaheer A., Rasheed A., Mukhtar M.K., Yaqoob R., Liaqat I. and Ahsan M.M. (2018) Silk of spider *Pholcus phalangioides* (Fuesslin, 1775) (Araneae: Pholcidae) as antibacterial agent. Acta Zoologica Bulgarica 70(4): 593-598.

Vierra C., Hsia Y., Gnesa E., Tang S. and Jeffery F. (2011) Silk composites and applications. Metal, Ceramic and Polymeric Composites for Various Uses. Intech Open. doi.org/10.5772/22894.

Vollrath F. and Krink T. (2020) Spider webs inspiring soft robotics. Journal of the Royal Society Interface 17: 20200569. doi.org/10.1098/rsif.2020.0569.

Wright S. (2011) The antimicrobial properties of spider silk. Master of Research Thesis, University of Nottingham. 1-113 pp.

Zhang S., Koh T.H., Seah W.K., Lai Y.H., Elgar M.A. and Li D. (2012) A novel property of spider silk: chemical defence against ants. Proceedings: Biological Sciences 279: 1824-1830. doi: 10.1098/rspb.2011.2193.

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