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Silk of the spider *Pholcus phalangioides* (Fuesslin, 1775) (Araneae: Pholcidae) as antibacterial agent

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Abstract: Spider silk has gained much attention in recent years because of its valuable properties. The present study is focused on the evaluation of the antibacterial properties of silk recovered from the cellar spider *Pholcus phalangioides* (Fuesslin, 1775) of the family Pholcidae. For the assessment of its inhibitory activities we used four pathogenic bacterial strains, i.e. two Gram negative (*Acinetobacter baumannii* and *Pasteurella multocida*) and two Gram positive (*Staphylococcus aureus* and *Streptococcus pneumoniae*). Antibacterial activity was evaluated following the Kirby-Bauer Disk diffusion method. The spider silk induced the highest inhibition zones against *A. baumannii* followed by *S. aureus* and *S. pneumonia.* The silk showed least effectiveness against *P. multocida*. Moreover, the inhibitory potential of silk varied with the concentration of the silk solution. On the basis of our results, we recommend further studies on the antibacterial action of spider silk, so that we may use silk of our local spider species for designing novel therapeutic agents in the near future.

Keywords: Spiders silk, drugs, antibacterial agent, *Pholcus phalangioides,* bacteria

Introduction

Nature has equipped spiders with one of the splendid artefact known as "Spider silk". The webs of spiders are specially fabricated to capture their prey and to save them from predators and external influences (Mishra et al. 2012). Apart from being crucial for spiders, spider silk has gained much attention in recent years for its potential to be utilised by mankind. Currently, the medical and pharmaceutical industries are drudging hard to develop an effective treatment against the prevailing infectious diseases. In this aspect, the antimicrobials derived from natural sources always surpass the synthetics because they include effective and eco-friendly therapeutic substances (Rosen et al. 2009).

In recent years, spider silk has attracted much attention as a substance with potent antibacterial activity due to the wealth of antimicrobial compounds (Roozbahani et al. 2014). Spider silk possess various hygroscopic amino acids, like glycine and alanine, which prevent it from desiccation. Moreover, it is also enriched with various other compounds, like potassium nitrate, bisphosphonate peptides (Gellynck et al. 2008), phospholipids hydrates and potassium hydrogen phosphate that have marked antibacterial activity (Heimer 1988, Gomes et al. 2010). Lipids present in the silk of *Linyphia triangularis* contain 12-methyltetradecanoic acid and 14-methylhexadecanoic acid that inhibit growth of microbes (Saravanan 2006). Spider coating peptides SCP-1 and SCP-2 are two important peptides found in the silk of *Latrodectus hesperus* (black widow spider). These peptides might be important for the antimicro-

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bial role of silk (Hu et al. 2005, Vierra et al. 2011). All of the above, when combined with future prospects, suggests that spider silk might serve as a lead compound for the development of new generation of antibiotics.

Various studies have revealed that spider silk is capable of potent inhibition of various types of bacterial strains. Recently, the spider silk recovered from the social spider, *Stegodyphus dumicola* is found to inhibit the growth of *Bacillus thuringiensis,* an entomopathogenic bacterium (Keiser et al. 2015). In another study conducted by Wright & Goodacre (2012), the silk of *Tegenaria domestica* is shown to have bacteriostatic effects against *Bacillus subtilis*, a Gram positive bacterium. Moreover, its silk does not seem to constrain the growth of mammalian cells, thus exhibiting remarkable potential for development of a promising therapeutic tool (Wright & Goodacre 2012). Also, the antimicrobial compounds of spider silk acquired from *Pholcus phalangioides* potently inhibit the growth of both Gram positive, i.e. *Listeria monocytogenes*, and Gram negative bacteria, i.e. *Escherichia coli* (Roozbahani et al. 2014). Mirghani et al. (2012) showed that the inhibitory effect of spider silk on *B. subtilis* was comparatively higher than on *E. coli*. Moreover, the silk recovered from *Lasiodora parahybana* has an evident inhibitory effect on the growth of Gram-positive bacteria (Wright 2011). Although, the spider silk of the orb-weaving spider, *Argiope aurantia* showed no significant inhibitory effects on growth patterns of three bacterial strains: *B. subtilis, E. coli* and *Pseudomonas aeruginosa*, it was resistant to adherence of the Gram negative bacteria (*E. coli* and *P. aeruginosa*) (Sharma 2014). Chakraborty & Das (2009) have also reported the anti-bacterial effect of spider silk.

The present study was specifically designed to evaluate the antibacterial potential of spider silk against standard drug resistant pathogenic bacteria. This study could provide baseline information for advanced antimicrobial drug development in the area.

Materials and Methods

Collection of spider silk

Common house spiders *Pholcus phalangioides* (Fuesslin, 1775) (family Pholcidae) were collected from walls, ceilings and attics using hand-picking. The collected spiders were maintained in the laboratory under controlled conditions: temperature 30°C and 75% humidity. Each spider was housed in separate container (4×4 feet), provided with physical supports to aid in web building. Each container was covered with a transparent plastic sheet with a centrally fitted fine mesh to ensure proper ventilation. The spiders were regularly fed with various flying insects including houseflies and crickets (Wright & Goodacre 2012). Inside the containers, the spiders produced silk under captive conditions. The woven silk was then collected on daily basis using sterile glass rods. These rods were gently run across the middle of the silk gossamers and instantly transferred into sterile jars to prevent any contamination.

Silk solution preparation

A total of about 300 mg of silk was recovered from the selected spider species. Various attempts were made to dissolve silk in different commonly used solvents like Acetone, sodium hydroxide Ethanol, SDS (Sodium Dodecyl Sulphate), Methanol and HCl. Only the 5% sodium hydroxide solution was capable of 80% dissolution of spider silk upon heating. So, to prepare standard stock solution of spider silk, 300 mg of silk was dissolved in 30 ml of 5% NaOH and heated for about 15 minutes at 100°C with constant shaking. This solution was then used as a 100% stock solution and 50% solution was prepared from it.

Culturing and maintenance of bacterial strains

In order to study the antibacterial potential of spider silk, two Gram negative (*Acinetobacter baumannii* and *Pasteurella multocida*) and two Gram positive (*Staphylococcus aureus* and *Streptococcus pneumoniae*) bacterial strains were secured from the Microbiology Laboratory, Department of Zoology, University of the Punjab, Lahore. These strains were cultured on nutrient agar. Afterwards, the bacterial colony of each strain was isolated from the culture medium and introduced in sterile test tubes containing 5 ml Brain Heart Infusion Broth. The test tubes were then labelled and incubated at 37°C for 24 hours and were stored at 4°C in liquid broth for further study.

Susceptibility test

Spider silk obtained from *P. phalangioides* was tested for its potential as an antibacterial agent by using the Kirby-Bauer Disk Diffusion Susceptibility Analysis Protocol. The formation of inhibition zone was considered an indication for the possession of antibacterial activity.

Agar plates preparation and assemblage

Nutrient agar medium was prepared in conical flask by dissolving 2 g nutrient agar, 0.3 g meat extract and 0.5 g peptone up to a final volume of 100 ml by

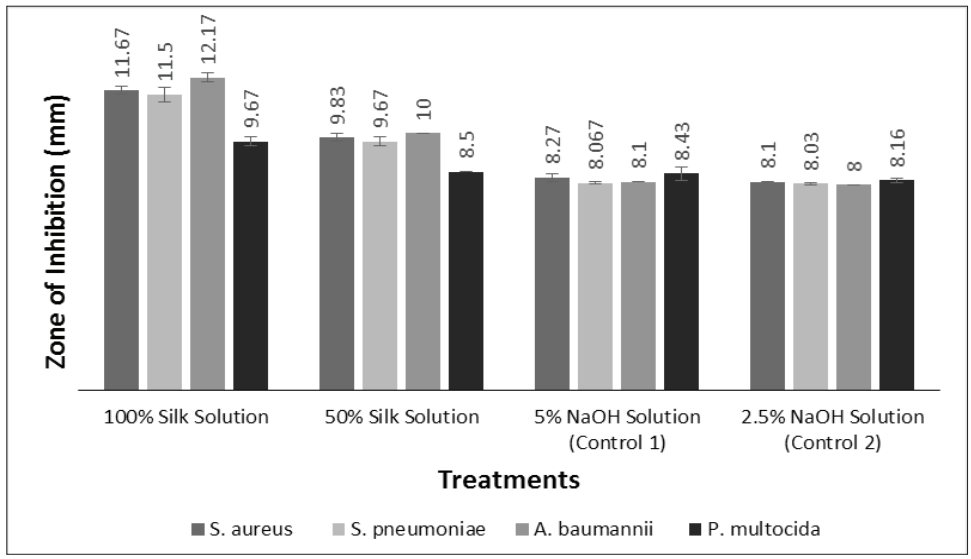

Fig. 1. Comparison of effect of the four treatments applied immediately after spreading colonies of bacteria on four bacterial strains.

Table 1. Comparison of inhibition zones (mm) produced with various treatments among different bacterial strains after 24 hours of incubation.*

Treatments	Bacterial strains			
	S. aureus	***S. pneumoniae***	***A. baumannii***	***P. multocida***
100% Silk Solution	11.67± 0.167^{c}	11.5± 0.28^{c}	12 .17± 0.17^{c}	9 .67± 0.167^{c}
50% Silk Solution	9.83± 0.167^{b}	9.67± 0.167^{b}	10^{b}	8.50^{b}
5% NaOH Solution (Control 1)	8.27± 0.15^{a}	8.067± 0.03^{a}	8.10^{a}	8.43± 0.27^{b}
2.5% NaOH Solution (Control 2)	8.10^{a}	8.03 ±0.03^{a}	8.00^{a}	8.16± 0.08^{ab}
$F_{4,10}$	163.09	100.06	608.26	56.89
P-value	< 0.001	< 0.001	< 0.001	< 0.001

*Values after ± represent standard errors of the mean and values in column, having different superscripts are significantly different. Results were recorded after 24 hours of incubation. Table should be read for Tukey's test column wise.

adding distilled water. The mixture was autoclaved for 30 minutes at about 120°C. The agar nutrient medium was cooled to 60-70°C and transferred in Petri dishes under aseptic conditions. These agar plates were left at room temperature to further cool down and solidify.

Spreading of bacteria on agar plates

For all bacterial strains 100 ul of bacterial suspension was pipetted and carefully tipped in the centre of separate agar plates. The bacterial suspension was then thoroughly spread on the agar plate with a sterile glass spreader. The spreading was followed immediately by the application of treatments.

On each agar plate, four treatments were applied. Two of them were considered as experimental treatments and the other two were applied as controls. The experimental treatments included 100 % and 50 % silk solutions, while 5 % and 2.5 % NaOH solutions were taken as control. For application of treatments sterilised discs of filter paper (8 mm diameter) were first dipped in the treatment solution and then dried a bit to get rid of excess solution. Then they were placed on agar plates by using sterile forceps. After that, the plates were incubated at 37°C for 24 hours. Inhibition zones (mm) were measured to evaluate the effectiveness of treatments. Moreover, to minimise the possibility of error, the experiment was run in triplicates at a time and repeated thrice for each treatment.

Statistical analyses

The mean and the standard error of the mean (Mean± SE) were computed using Minitab 14. Normality of data was asses using the Kolmogorov–Smirnov test (Sherawat et al. 2015). One-way ANOVA followed

by Tukey's test was applied to compare the zone of inhibition of the four groups using SPSS version 13 (Statistical Package for Social Sciences, 13).

Results

The inhibition zones in control groups were significantly smaller than the silk-treated. Tukey's test multiple comparisons are given in Table 1. The maximum zone of inhibition was observed when *A. baumannii* was treated with 100 % silk solution ($F_{3,8}$; F=608.26; P< 0.001). The inhibition zone was reduced by reducing silk concentration. Similar results were obtained against *S. aureus* ($F_{3,8}$; F=163.09; P< 0.001) and *S. pneumoniae* ($F_{3,8}$; F=100.06; P< 0.001). However, in the case of *P. multocida* ($F_{3,8}$; F=56.89; P< 0.001), overall difference among treatments was significant ($F_{3,8}$; F=56.89; P< 0.001). Multiple comparisons indicated that only the inhibition zone formed due to 100% silk solution for *P. multocida* was statistically different from the other treatments, while the difference among the other three treatments was non-significant (Table 1). Furthermore, it is evident from Table 1 that the maximum zone of inhibition among silk-treated groups was recorded against *A. baumannii* (i.e., 12.17± 0.17), followed by *S. aureus* (i.e., 11.67± 0.167), *S. pneumoniae* (11.5± 0.28) and *P. multocida* (9.67 ± 0.167; Fig. 1).

Discussion

Our results revealed the significant inhibitory effect of *Pholcus phalangioides* silk against *A. baumannii, S. aureus, S. pneumonia* and *P. multocida*. Many researchers have discussed the possibility that spider silk can inhibit growth in microbes (Heimer 1988, Fairbrother et al. 1990). But only few scientists have tested and verified it. One such study concerning antimicrobial nature of spider silk is conducted by Roozbahani et al. (2014) which implies that the silk obtained from *P. phalangioides* has significant inhibitory effect on both Gram negative (*E. coli*) and Gram positive (*L. monocytogenes*) bacteria. Similar were the results reported by Mirghani et al. (2012) who showed significant growth inhibition in both *E. coli* and *B. subtilis* after treatment with silk solution.

Anti-bacterial effects of spider silk against both Gram negative and Gram positive strains have also been reported by Chakraborty & Das (2009). The egg sac silk of *Pityohyphantes phrygianus* also exhibit an inhibitory effect on the growth of *B. subtilis* and *E. coli*. Amaley et al. (2014) reported that the silk of *Nephila pilipes* possess antibacterial activity against *E. coli* and *P. aeruginosa*, as well as against *S. aureus*. These findings are in accordance with our results, indicating that the spider silk is potent for both Gram negative and Gram positive strains. The measured inhibition zone in our experiment was much bigger as compared to that reported by the other researchers (Mirghani et al. 2012, Wright & Goodacer 2012, Sharma 2014). The reason might be the use of raw or partially dissolved silk in the previous studies. We have used 5 % NaOH to dissolve about 80 % of the silk.

On the other hand, some studies have also led to the conclusion that spider silk is capable of effective inhibition of only Gram positive strains. A study conducted by Wright & Goodacre (2012) has revealed that the web silk of *T. domestica* can inhibit the growth of the Gram positive bacterium *B. subtilis*, while the silk of the same specie was found ineffective against *E. coli*. The web silks of *Zilla diodia* and *Linyphiidae* spiders showed an inhibitory effect on the growth of only *B. subtilis*. Furthermore, the silk of *L. parahybana* is also known to possess some antimicrobial activity against Gram positive bacteria (Wright 2011). This disagreement could be explained by the fact that the antibacterial activity of spider silk recovered from different spider species varies with the attributes, quality, type and arrangement of amino acids in the silk fibre (Wright & Goodacre 2012). Furthermore, the antimicrobial potential of spider silk may also alter with the selected microbial species.

According our results, spider silk treatment is effective against all Gram negative and Gram positive aerobic bacterial species. However, a reduced trend of inhibition was recorded against *P. multocida,* an anaerobic Gram negative bacteria. This deviation may suggest that our experimental set up was not sensitive enough to detect significant inhibitions against this microbe or it could be possible that like many other antimicrobial agents, our treatment (silk of *P. phalangioides*) has narrow spectra of activity and generates differential inhibitions in different types of bacteria. The difference in inhibition of bacteria could also be explained by the species phylogenetic distance. Most importantly there is a possibility that the silk treatment has reduced coverage on anaerobic strains. A study conducted by Wright & Goodacre (2012) has revealed that the web silks of *T. domestica*, *Z. diodia* and *Linyphiidae* spiders were either ineffective or has reduced activity against *E. coli*. Mirghani et al. (2012) and Roozbahani et al. (2014) also reported reduced inhibitory activity of silk against *E. coli*. Amaley et al. (2014) observed no inhibition zone around dra-

gline silk samples in the culture of *Klebsiella pneumoniae*, a Gram negative bacterium.

Our study also reveals that the spider silk is potently more inhibitive for the growth of aerobic Gram negative bacterial strains as compared to Gram positive ones. We found that the largest zones of inhibitions were recorded in *A. baumannii* followed by *S. aureus* and *S. pneumoniae* bacterial strains. Similar results were reported by Chakraborty & Das (2009), who explained that the proteins separated from the web silk of the common house spider *Crossopriza lyoni* induced significant inhibition zones with much lower MIC (Minimum inhibitory concentration) values (<10 mcg/mL) in Gram negative bacteria (*E. coli* and *P. aeruginosa*) than those in Gram positive bacteria (*S. aureus* and *Enterococcus faecalis*). Our findings are also closely related to the studies of Sharma (2014) and Amaley et al. (2014). Sharma (2014) demonstrates that the surface of spider silk shows low adherence to Gram negative bacteria (*P. aeruginosa* and *E. coli*) as compared to Gram positive ones (*B. subtilis*). This is because spider silk surface is glazed with several antibacterial fatty acids, such as polyunsaturated 12-methyltetradecanoic acid and non-protein amino acids like GABA (Gamma-amino-butyric acid), preventing attachment of Gram negative bacteria. Amaley et al. (2014) reported greater inhibitory effect of the web silk of *N. pilipes* against *P. aeruginosa* and *E. coli* in comparison of *S. aureus*.

Our results suggesting that the spider silk is more potent against Gram negative strain contradict the findings of Mirghani et al. (2012) who reported a slightly greater inhibition zones in *B. subtilis* as compared to *E. coli*. Similar results were established by Roozbahani et al. (2014) while working with *L. monocytogens* and *E. coli*. The study of Wright & Goodacre (2012) indicated that only *B. subtilis* is susceptible to the inhibitions induced by spider silk. This can be again in explained by the differential antimicrobial activity of spider silk recovered from different spider species and the phylogenetic distances of the selected bacterial strains.

Conclusion

Spider silk has antibacterial potential and its applications should be studied further. Antimicrobial compounds obtained from spiders have created the hope for the effective treatment of emerging bacterial infections. Addressing the safety concerns of new therapies to biological systems is currently needed.

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References

Amaley A., Gawali A. A., & Akarte S. R. 2014. Antibacterial nature of dragline silk of *Nephila pilipes* (Fabricius, 1793). Indian Journal of Arachnology 3: 8.

Chakraborty D. & Das S. 2009. Antibacterial activities of cobweb protein. 19th ECCMID (European Congress of clinical microbiology and infectious diseases), Helsikni, Finland. Abstract No. R2127.

Fairbrother W. J., Graham H. C. & Williams R. J. P. 1990. The roles of ATP4− and Mg2+ in control steps of phosphoglycerate kinase. Federation of European Biochemical Societies 190: 407–414.

Gellynck K., Verdonk P. C., Vannimmen E., Almqvist K. F., Gheysens T., Schoukens G., Vanlangenhove I., Kiekens P., Mertens J., & Verbruggen G. 2008. Silkworm and spider silk scaffolds for chondrocyte support. Journal of Materials science: Materials in medicine 19: 3399

Gomes S., Leonor I. B., Mano J. F., Reis R. I. & Kaplin D. I. 2010. Functionalized silk biomaterials for bone regeneration. Semana de engenharia 2010: 1–2.

Heimer S. 1988. Wunderbare Welt der Spinnen, Urania. Verlag Leipzig Jena Berlin (Urania) ISBN 10: 3332002104.

Hu X., Lawrence B., Kohler K., Falick A. M., Moore A. M., Mc Mullen E., Jones P. & Vierra C. 2005. Araneoid egg case silk: a fibroin with novel ensemble repeat units from the black widow spider, *Latrodectus hesperus*. Biochemistry 44(30): 100-207 .

Keiser C. N., Demarco A. E., Shearer T. A., Robertson J. A. & 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.

Mirghani M. E. S., Kabbashi, N. A., Elfaki, F. A., & Zulkifli M. Z. F. B. 2012. Investigation of the spider web for antibacterial activity. Malaysian International Conference on Trends in Bioprocess Engineering 201: 1–5.

Mishra R., Ahmad G., & Chaubey, S. N. 2012. Study on the morphology, feeding capacity and prey preference of *Neoscona crucifera* and *N. adianta* (Orb-Weaving Spiders). Indian Journal of Life Sciences 1: 29–34.

Roozbahani H., Asmar M., Ghaemi N. & Issazadeh K. 2014. Evaluation of Antimicrobial Activity of Spider Silk *Pholcus Phalangioides* against Two Bacterial Pathogens in Food Borne. International Journal of Advanced Biological and Biomedical Research 2: 2197–2199.

Rosen J., Gottfries J., Muresan S., Backlund A. & Oprea T. I. 2009. Novel chemical space exploration via natural products. Journal of Medicinal Chemistry 52: 1953–1962.

Saravanan D. 2006. Spider silk-structure, properties and spinning. Journal of Textile and Apparel, Technology and Management 5:1-20.

Sharma S. 2014. A study on spiders as predators in the Agro Ecosystems. Munis Entomology & Zoology Journal 9: 80–83.

Sherawat S. M., Butt A. & Tahir H. M. 2015. Effects of pesticides on agrobiont spiders in laboratory and field. Pakistan Journal of Zoology 47(4): 1089-1095.

Vierra E., Hsia Y., Gnesa E., Tang S. & Jeffery F. (2011). Spider Silk Composites and Applications. In: Metal, Ceramic and Polymeric Composites for Various Uses, Dr. John Cuppoletti (Ed.), InTech, DOI: 10.5772/22894. Available from: http://www.intechopen.com/books/metal-ceramic-and-polymeric-composites-for-various-uses/spider-silk-composites-and-applications

Wright S. 2011. The antimicrobial properties of spider silk. Thesis submitted to the University of Nottingham.

Wright S. & Goodacre S. L. 2012. Evidence for antimicrobial activity associated with common house spider silk. BMC Research Notes 5: 326.

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