WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 8, Issue 11, 106-109. Review Article ISSN 2277– 7105

ANTIMICROBIAL ACTIVITY OF EXTRACTS OF SPIDER WEB SILK

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Article Received on
08 August 2019,

Revised on 28 August 2019,
Accepted on 18 Sept. 2019,

DOI: 10.20959/wjpr201911-15657

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ABSTRACT

Spider silk extracts in in various solvents with different polarities were prepared and antibacterial and antifungal actives were carried out. Four bacterial strain bacillus subtillus, staphylococcus aureus, Escherichia coli, and pseudomonas aeruginosa and four fungal strains including pencillum digitatum, aspergilus niger, fusarium solani and aspergillus flavus were used while ampicillin 1000µg/ml and nystatin (8µg/ml) were used as control in anti-bacterial and anti-fungal activities respectively. Spider silk did not show any activity against *P. digitatum* and *A.flavus.* But different extracts of spider silk resulted different activities against *Fusarium solani* and *Aspergillus Niger*. The maximum antimicrobial activity of spider silk extract was measured in acetone against *B.subtilis*, in ethyl acetate against *E.coli*, in methanol against *P.aeruginosa* and in ethyl acetate against S.aureus at 24 hour.

INTRODUCTION

Spiders are a cause of plotting dread and numerous mythologies, but it is extraordinary to see that regardless of environment the spiders are found almost in every kind of habitation range.[1] Spiders have a life span of years and are famous for their sturdiness.[2] Spiders are among the most common specie found on the earth with such a great diversity.[10] They are fortified with nature's astounding material known as "Spider silk". Silk production is evolution in arthropods.[7] Spider silk suggest promising venues for the next decade of research on fascinating creation and its snare.[3 & 4] About 47,188 species of spiders have been acknowledged, out of which 41,000 species are categorized as silk producing[5 & 6], and evolution of spiders has only left 4600 species that produce orb web. The webs of spiders are especially made-up to catch their prey, in addition to that spider web is also a home and protection for spiders.[13] Composition of silk may differ because of habitat, food, and life

style, some spiders produce different kinds of silk at a same time.[14] Spiders not only helps in decreasing the number of pests and harmful insects but also stabilizes the ecosystem and food chain. Size of spider vary.[12] Web range from < 2cm (for example *Anapidae)* to almost 2m (for example *Caerostris darwini*) in diameter.[9&11] Spiders can produce up to seven different kinds of silk.[15&16] Spider silk is a commercially desired biomaterial due to possession of extraordinary properties such as high tensile strength, utmost elasticity, extreme hardness bio medical, bio-engineering, environment cleaning and therapeutic applications. It is five times stronger than steel and two times that of Kevlar.[17] Mechanical properties of silk can be correlated with its composition, glycine rich regions and polyalanine segments are the basic factors which influence high tensile strength and extensibility, which is why some spider silk types can stretch up to 140% of their original length without breaking.[19] Studies have revealed that at very low temperature (-196 °C) strength of silk improved by 64% as related to strength at room temperature.[20] Unique and extraordinary mechanical properties makes spider silk superior than the man-made artificial fibers.[21] It is used in manufacturing of rust free panels on boats, motor vehicles bullet proof jackets, furthermore, biodegradable bottles, bandages surgical thread, earth quake proof construction material, ropes of elevators, bridges and pillar might also be the potential items made out of the spider silk.[18]

MATERIALS AND METHODS

Web silk was procured from Bhatta Chowk Bedian Road Mananwala Lahore Cantt, during November 2017. Sample was cleaned manually and washed using distilled water. Sample was dried under shade and different pickets of web silk were made and kept in closed container for further processing. Different extracts by dipping dry web silk for about 48 hours using DCM, Diethyl ether, chloroform, ethyl acetate and acetone as the solvents. The weighed silk (25g) was dipped in 100ml (0.10 lit) solvent in the brown glass sealed jar. It was filtered off, and pure extract was stored in sealed bottle. Methanol extract was obtained through Soxhlet extractor.

The agar disc diffusion method was employed for determination of antibacterial and antifungal activity of different extracts of spider silk, following the procedure of bayder *et al* against different food borne pathogens including bacteria (gram positive, *Bcillus saubtilis, Staphylococcus aureus and gram negative, Escherichia coli, Pseudomonas aeruginosa*) and fungi (*Aspergillus falvis, Pencillum digitatum, Fusarium solani and Asperigillus niger*). Standard culture were obtained from Pakistan Type Culture Collection (PTCC) standard

culture media (CM69, CM139, CM271, CM145, CM201, CM7) from oxide were employed throughout the present investigation for the purpose of culture maintenance at the respective temperature 25ºC for fungi and 37ºC for bacteria.

Sterile and dried 4mm thick paper discs (difco) were impregnated with filter sterilized 0.45mm Millipore filler newly extracted oil and placed on freshly seeded fungal lawn 7 discs in a plate with a control. All experiments were conducted in triplet. The petri plates were incubated and zone of inhibition was calculated in millimeters after 24 hours.

Anti-microbial assay

Agar disc diffusion method was used for the estimation of antimicrobial activity of spider silk in different solvents. Eight food borne pathogenic specimens were included in study. Standard culture is obtained from Pakistan type culture collection. Sterile and dried 4mm thick paper defco was used for diffusion.

Table 1: Antibacterial and antifungal activity of various extracts of spider web silk.

Tested Microorganism	Incubation temp.(°C)& culture media	Colony Morphology	Methanol extract	Diethyl ether extract	DCM extract	Chloroform extract	Ethyl acetate extract	Acetone extract	Control inhibition (mm)
Bcillus subtilis	37 °C- CM7	Gram + ve Rods	6.5	-	6.5	8	7	10	28
Escherichia Coli	37 °C- CM69	Gram -+ve Rods	6	-	9	8.5	10	7	40.1
Staphylococcus aureus	37 °C- CM145	Gram + ve cocci	6	6	6	-	-	6	18
Pseudomonasaeruginosa	37 °C-CM7	Gram -ve Rods	6.5	-	6.5	8	7	-	16
Pencillum digitatum	25 °C- CM139	White later cottony	-	-	-	-	-	-	24.0
Aspergillus Niger	25 °C- CM139	Black later white	20	5	7	10	9	18	25
Fusarium solani	25 °C- CM139	White later white dots	8	11	8	8.5	8.5	10	22
Aspergillus flavus	25 °C- CM139	White later white dots	-	-	-	-	-	-	25

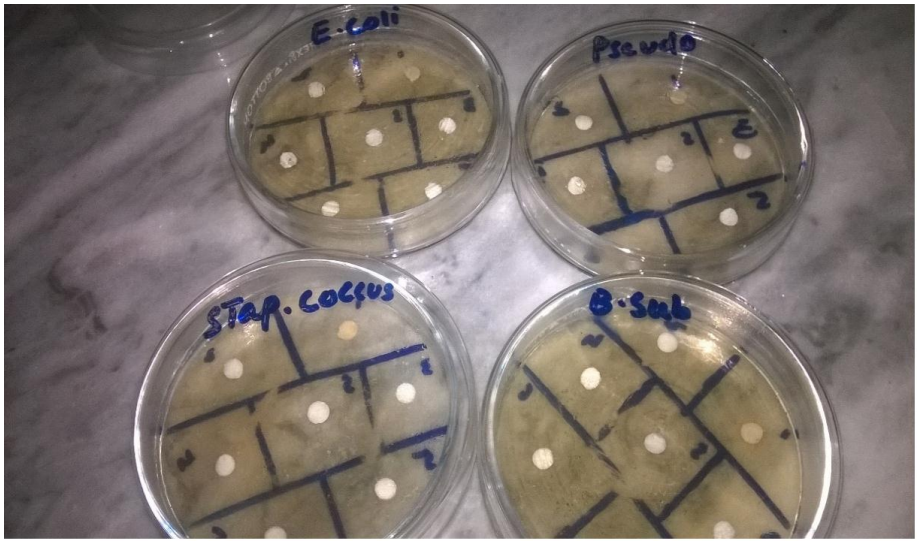

Fig 4.5: Anti bacterial activity after 24 hours.

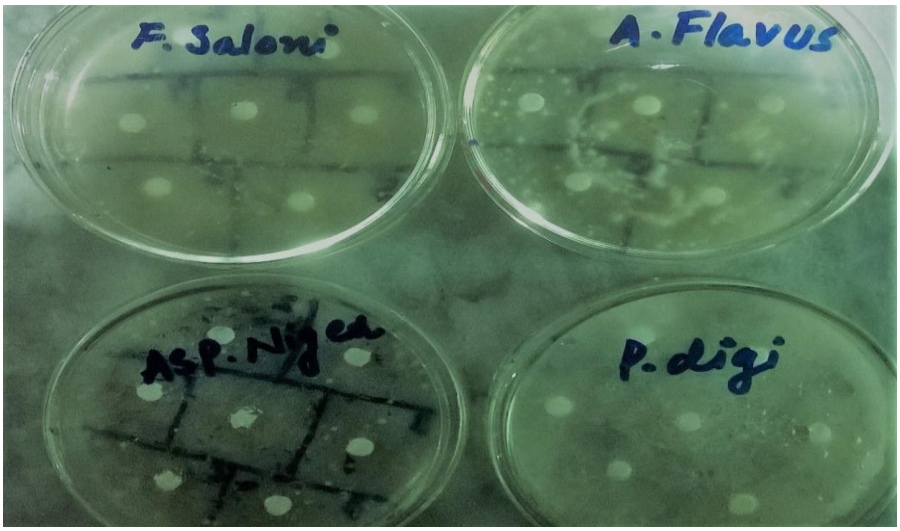

Fig 4.6: Anti-fungal activity after 24 hours.

In this study we demonstrate that some silk types likely do inhibit bacterial growth, although only certain types of bacteria may be vulnerable. The strongest evidence supporting the view that silk has antibacterial properties comes from work on silk from *Tegenaria domestica*, the common house spider. This species belongs to the Agelenidae and uses its silk for prey wrapping and constructing funnel webs. The studies appear to show that silk of spiders may have antimicrobial properties. There appears to be activity against gram positive and gram negative, although the effect does not appear long lasting and 48 hours after the start of the experiment, no activity against microbes can be detected. There appears to be no similar effect against fungi. Bacteriostatic is activity of organic component that lowers rate of bacteria to grow and reproduce.[19] Comparative study was also done on *Tegenaria domestica*

silk in 2012 on *B.subtilis* (gram positive bacteria) and *E.coli* (gram negative bacteria), spectrophotometer was used to measure microbial activity, and result concluded was that spider silk is bacteriostatic in nature on *B. subtilis* but no significant effect on gram negative bacteria.[20] Argiope dragline silk against gram positive and gram negative bacteria does not show any bactericidal action, but prevents bacterial adherence.[21] Sipder silk oil was extracted in methanol, DCM, Diethyl ether, chloroform, ethyl acetate and acetone. These extracts were studied against different bacterial strains (gram positive, Bcillus saubtilis, Staphylococcus aureus and gram negative, Escherichia coli, Pseudomonas aeruginosa) and fungal strains (Aspergillus falvis, Pencillum digitatum, Fusarium solani and Asperigillus niger).

The high amount of variation in the data meant that the inhibitory effect of the silk was not shown on every single sample. When examined after 24 hours of growth there was a trend in the samples with silk present showing less growth of bacteria.

Acetone and chlororform extracts give more zone of inhibition as compared to other extracts in case of B. subtilis, while in case of E.coli Ethyl acetate >DCM >Chloroform give more and clear zone of inhibition. Against Pseudomonas aeruginosa only methanol extracted oil give clear zone of inhibition, while in case of Staphylococcus aureus except ethyl acetate and chloroform all the extract give small zone of inhibition. Ampicillin 1000µg/ml was used as control. Spider silk against fungal strains does not give similar results as compared to the bacterial strains. Antimicrobial peptides create an acidic environment above pH of 4 which is not suitable for fungal growth.[21] Two species of fungus, *S. Cerevisiae* and *A. Niger* were tested against silk. Average growth of fungus with silk was lower but no significant results were deduced. It was estimated either these fungus types are resistant to silk or bacteria present are masking the antifungal properties. Anecdotal evidence suggest cobwebs are usually not decomposed by fungus or bacteria.[20] Against some strains sample show positive result but after 24 hours no antifungal activity was detected. Spider silk does not show any activity against *Pencillum digitatum* and *Aspergillus flavus.* While against *Fusarium solani* diethyl ether extract and acetone extract and against *Aspergillus Niger* methanol extract and acetone extract give most positive results. Nystatin (8µg/ml) was used as control in antifungal activity. Results show that action of spider silk oil is specific against fungus, against some fungal strain result was positive and against some strains was negative. Thus, it could be used against specific fungal strains.

REFERENCES

1. Uetz, G. W. (1991). Habitat structure and spider foraging. In Habitat structure (pp. 325-348). Springer, Dordrecht.
2. Blackledge, T. A., & Gillespie, R. G. Convergent evolution of behavior in an adaptive radiation of Hawaiian web-building spiders. Proceedings of the National Academy of Sciences of the United States of America, 2004; 101(46): 16228-16233.
3. Blackledge, T. A., & Hayashi, C. Y. (2006). Silken toolkits: biomechanics of silk fibers spun by the orb web spider Argiope argentata (Fabricius). Journal of experimental biology, 1775; 209(13): 2452-2461.
4. Agnarsson, I., Kuntner, M., & Blackledge, T. A. Bioprospecting finds the toughest biological material: extraordinary silk from a giant riverine orb spider. PloS one, 2010; 5(9): e11234.
5. Platnick, N. I. (2011). The world spider catalog, version 11.5. http://research. amnh. Org/entomology/spiders/catalog/index. html.
6. Platnick, N. I., & Dupérré, N. The Andean goblin spiders of the new genus Scaphidysderina (Araneae, Oonopidae), with notes on Dysderina. American Museum Novitates, 2011; 3712: 1-51.
7. Bram, A., Bränden, C. I., Craig, C., Snigireva, I., & Riekel, C. X-ray diffraction from single fibres of spider silk. Journal of applied crystallography, 1997; 30(3): 390-392.
8. Schulz, S. Composition of the silk lipids of the spider Nephila clavipes. Lipids, 2001; 36(6): 637-647.
9. Coddington, J. A., & Levi, H. W. Systematics and evolution of spiders (Araneae). Annual review of ecology and systematics, 1991; 22(1): 565-592.
10. Lin, L.H.,.T Edmonds, D. And Vollrath, F. Structural engineering of an orb-spider's. Nature, 1995; 373: 146.
11. Blackledge, T. A., Scharff, N., Coddington, J. A., Szüts, T., Wenzel, J. W., Hayashi, C. Y., & Agnarsson, I. Reconstructing web evolution and spider diversification in the molecular era. *Proceedings of the National Academy of Sciences*, 2009; *106*(13): 5229-5234.
12. Gregorič, M., Agnarsson, I., Blackledge, T. A., & Kuntner, M. Darwin's bark spider: giant prey in giant orb webs (Caerostris darwini, Araneae: Araneidae), *Journal of Arachnology*, 2011; *39*(2): 287-295.

13. Mishra, R., Ahmad, G. and Chaubey, S.N. Study on the morphology, feeding capcity and prey preference of Neoscona crucifera and N. adianta (orbweaving spiders). Indian J. L. Sci., 2012; 1: 29-34.
14. Foelix, R.F., 1996. Biology of spiders. 2nd Edition, Oxford University Press, New York
15. Work, R. and Young, C. The amino acid compositions of major and minor ampullate silks of certain orb-webbuilding spiders (Araneae, Araneidae)." J. Arachnol., 1987; 15(1): 65-80.
16. Craig, C.L. "Evolution of arthropod silks." Ann. Rev. Entomol., 1997; 42: 231-267.
17. Vollrath, F. And Knight, D.P. Liquid crystalline spinning of spider silk. Nature, 2001; 410(6828): 541-548.
18. Singha, K., Maity, S. and Singha, M., Spinning and applications of spider silk. Front. Sci., 2012; 2: 92-100.
19. Liévin-Le Moal, V., & Servin, A. L. The front line of enteric host defense against unwelcome intrusion of harmful microorganisms: mucins, antimicrobial peptides, and microbiota. *Clinical microbiology reviews*, 2006; *19*(2): 315-337.
20. Wright, S., & Goodacre, S. L. Evidence for antimicrobial activity associated with common house spider silk. *BMC research notes*, 2012; *5*(1): 326.
21. Pankey, G. A., & Sabath, L. D. Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. *Clinical infectious diseases*, 2004; *38*(6): 864-870.