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Short Communication

ANTI-BACTERIAL POTENTIAL OF SILK RECOVERED FROM *ERIOVIXIA EXCELSA* (SIMON, 1889) SPIDER

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ABSTRACT

Present study was designed with an aim to evaluate anti-bacterial potential of an orb-web spider *Eriovixia excelsa.* Live spiders were collected from the field and reared in the laboratory for harvesting silk. Disc diffusion methods was used to evaluate antibacterial potential of silk. Silk of *E. excelsa* showed negligible antibacterial activity against *Pasteurella* sp. and *Staphylococcus* sp. at all tested concentrations (i.e., 100%, 75%, 50% & 25%). However, spider silk inhibited the growth of *Streptococcus* sp. and *Acinetobactor* sp. Inhibitory potential of silk was increased with the increase in silk concentration. ANOVA followed by Tukey's test revealed significant difference among treatments. It is concluded that silk of *E. excelsa* could be used to prepare antibiotics against *Pasteurella* sp. and *Staphylococcus* sp.

Key words: Spider, Silk, Antibacterial potential, Disc diffusion, *Eriovixia excelsa*.

INTODUCTION

The pharmaceutical industry is currently facing the trouble in developing effective antimicrobials for prevailing infectious diseases. The dilemma has been worsened by the selective pressure exerted by extensive use and misuse of synthetic antimicrobials (Witte, 2000). The pathogens are becoming resistant more quickly than the discovery of new drugs. Thus, the alarming problem of drug resistance is of particular concern and should be addressed on immediate basis. Therefore, the search for new antimicrobial approaches seems to be imperative that could better serve the medicinal industry to develop biologically safer antibiotics.

The antimicrobials derived from natural sources have always been regarded superior to synthetics in the scientific community because they occupy a broad range of biologically relevant and bio-friendly therapeutic chemicals (Rosén *et al.,* 2009). Spider silk components are a source of hope to overcome the current barriers in the development of curative agents with desirable properties. Exclusive properties of silk such as biocompatibility, thermal stability, controllable degradation and surface chemistry make it appropriate for many biomedical applications (Vepari and Kaplan, 2007; Chau, 2008).

Spider silk is mainly composed of Glycine, Alanine and large amount of pyrrolidine. These amino acids maintain the moisture of spider silk and prevent it from drying out. Silk becomes acidic due to presence of these amino acids and microbes are unable to grow on it due to its acidic nature. Acidic environment prevent the biofilm formation and proves a challenging environment for bacterial growth (Cotter and Hill, 2003; Dagorn *et al.,* 2013). Potassium nitrate present in silk also inhibit the growth of microbes on silk (Chakraborty and Das, 2009; Gomes *et al.,* 2010).

Spider silk is the current focus of interest of many scientists as potential antimicrobial agent. Efforts are being made in the medical field to exploit the antimicrobial potential of spider silk to develop novel antibiotics. Spider silk has been found to resist bacterial attachment on its surface owing to its microbe-repellent properties (Gomes *et al.,* 2010; Zhang *et al.,* 2012). Present study was designed to evaluate the antibacterial potential of silk harvested from *Eriovixia excelsa* against four bacterial strains i.e., *Acinetobactor* sp., *Pasteurella* sp., *Staphylococcus* sp., and *Streptococcus* sp. *E. excelsa* is an orb-weaving spider of family Araneidae which is commonly found in citrus orchards.

MATERIALS AND METHODS

Spider collection: Live *Eriovixia excelsa* (Simon, 1889) spiders were collected from citrus orchards of district Sargodha, Punjab, Pakistan by hand picking and jerking methods (Tahir *et al.,* 2015). Especially designed wooden boxes (4/4 feet) were used to house spiders. Spiders were allowed to build webs in these boxes. Houseflies and plant hoppers were provided as food source to the spiders on daily basis.

Harvesting of silk: Sterile glass rods were used for the collection of silk from the boxes. Sterile glass rods with attached silk were placed in sterile glass box to avoid any

contamination. Silk from the boxes was collected on daily basis.

Preparation of silk solution: 1.5g spider silk (1.5g) was dissolved in NaOH (2.5%) and heated for 7-10 minutes. The resultant mixture was considered as stock solution (100% solution). Four concentrations i.e., 75%, 50% and 25% from stock solution were prepared. To prepare 75% dilution, 7.5ml of stock solution was mixed with 2.5ml distilled water. To prepare 50% dilution, 5ml of stock solution was mixed with 5ml of distilled water and to prepare 25% dilution 2.5ml of stock solution was mixed with 7.5ml of distilled water. Dilutions (*i.e.,* 100%, 75%, 50%, and 25%) of 2.5% NaOH were also prepared to be used as control treatments.

Bacterial collection and storage: To investigate antibacterial activity of spider silk selected Gram negative (*Acinetobactor* sp.) and Gram positive (*Pasteurella* sp., *Staphylococcus* sp., and *Streptococcus* sp.) bacterial strains were used. All strains of bacteria were obtained from Microbiology Laboratory, Department of Zoology, University of the Punjab, Lahore. Bacterial strains were cultured on agar plates at 37˚C. Isolated colony of each bacterial strain from the agar plate was transferred to 5mL of autoclaved liquid broth using sterile loop. Tubes with inoculated broth were incubated at 37˚C for 24 hours. Subsequently, all bacterial strains were stored in brain heart infusion (BHI) broth at 4˚C. The broth medium was prepared by dissolving 3.7g of BHI powder in 100ml of distilled water followed by autoclaving at 121˚C for 15 minutes at 15 lb pressure.

Assessment of antibacterial activity: Antibacterial potential of spider silk was evaluated using Kirby-Bauer Disk Diffusion Susceptibility Analysis Protocol and zones of inhibition were measured in millimeter. To perform the test, nutrient agar plates were prepared and stored upside down in a refrigerator at 4˚C. The 100 µl bacterial suspension of approximately 1–2×10^6CFU/mL () was spread on the surface of agar using sterile glass spreader. Sterile filter paper discs of 6mm diameter were placed on the surface of the agar plates at appropriate distance.

Five filter paper discs were applied on each inoculated agar surface plate. One disc placed at the centre served as control and other four were considered as experimental treatment. Afterwards, 25µl of 100% silk solution was poured on each of the four experimental filter paper discs. Whereas, 25µl of 100% NaOH was poured on centre disc, referred as control in this study. The same procedure was repeated for other concentrations *(i.e*., 75%, 50% and 25%).

Plates were incubated for 24 hours at 37˚C. After incubation, inhibition zone was observed and measured to assess the effectiveness of antibacterial potential of spider silk. Diameter of inhibition zone was measured in millimeter. The experiment was replicated thrice to minimize the possibility of error.

Statistical Analyses: Normality of the data was assessed before application of any statistical analysis. Data was presented as Mean and standard error of mean (Mean±SE). One way Analysis of variance followed by Tukey's test was used to compare the effect of treatments on zone of inhibition.

RESULTS

The silk of *Eriovixia excelsa* showed negligible antibacterial activity against *Pasteurella* sp. and *Staphylococcus* sp. at all tested concentrations (i.e., 100%, 75%, 50% & 25%) of spider silk (Table 1). However, spider silk inhibited the growth of *Streptococcus* sp. and *Acinetobactor* sp. Significantly prominent zones of inhibition for these bacterial strains were observed at 24 hours of post treatment. The zone of inhibition was increased with the increase of silk concentration (Table 1).

Table 1. Comparison of inhibition zones (mm) obtained with application of 25 µl of four different concentrations of spider silk solution against *Acinetobactor* sp.

	Bacterial strains			
Conc. of silk solution	***Acinetobactor* sp.**	***Streptococcus* sp.**	***Pasteurella* sp.**	**S*taphylococcus* sp.**
100%	22.33^{d} ±0.33	19.67^{d} ±1.20	0.31^{a} ±0.11	0.29±0.0.08
75%	19^{c} ±0.57	17.33^{c} ±0.88	0.29^{a} ±0.13	0.23±0.11
50%	16.66^{c} ±0.88	16.33^{c} ±0.66	0.29^{a} ±0.09	0.21±0.0.07
25%	8.33^{b}±0.33	12^{b} ±1	0.24^{a} ±0.14	0.19±0.10
Control	0.20^{a} ±0.33	0.18^{a} ±0.33	0.22^{a} ±0.11	0.20±0.0.9
DF	*4,10*	*4,10*	*4,10*	*4,10*
F	*106.97*	*20.98*	*106.97*	*20.98*
P-value	*<0.001*	*<0.001*	*>0.05*	*<0.05*

Note: *Values after ± in above table are representing standard error of mean and values having different superscripts are significantly different. Results were recorded after 24 hours of incubation.*

For *Acinetobactor* sp. significant differences were observed in the zones of inhibition among treatments ($F_{4,10}$=*106.97; P=<0.001*). Results of Tukey's test confirmed that the zone of inhibition with minimum silk concentration (25%) was significantly smaller than the zone of inhibition produced with 100% silk (Table 1). Furthermore, it is also evident from Table (1) that zones of inhibition produced with 50% and 75% did not differ statistically. Statistically significant differences were also observed in zones of inhibition when 25µl of four different concentrations (i.e., 100%, 75%, 50% and 25%) of spider silk were applied against *Streptococcus* sp. ($F_{4,10}$=*20.717; P=<0.001).*

DISCUSSION

Results of our study revealed that silk of *Eriovixia excelsa* possess inhibitory effect against *Acinetobactor* sp. and *Streptococcus* sp. after 24 hours of incubation period. No antibacterial activity was recorded against *Pasteurella* sp. and *Staphylococcus* sp. Spider silk was found to be more effective against Gram negative bacteria (*Acinetobactor* sp.) than Gram positive bacteria (*Streptococcus* sp.). Surface of spider silk is coated with glycoproteins that are about 150-250nm thick (Augsten *et al.,* 2000). An antibacterial property of spider silk is due to glycoproteins (Wright and Goodacre, 2012). The antibacterial nature of silk is advantageous for spiders because they store their food for months and even years in a packaging of silk without being attacked by bacteria or fungus (Eberhard *et al.,* 2006). This preservative property of silk is due to the presence of antimicrobial compounds in the spider silk (Roozbahani *et al.,* 2014).

Results of our study are in accordance with Amaley *et al.* (2014) who proved that the silk of *Nephila pilipes* possess antibacterial activity against *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa.* According to their findings, silk of *Nephila pilipes* shows more inhibitory effect against *P. aeruginosa* and *E. coli* (Gram negative bacteria) than *S. aureus* (Gram positive bacteria). Sharma (2014) also reported that spider silk shows low adherence to Gram negative bacteria (*E. coli* and *P. aeruginosa)* as compared to gram positive bacteria *(B. subtilis).* Lipopolysaccharides present in cell wall of Gram negative bacteria play a fundamental role in their attachment to any biotic or abiotic surface (Donlan, 2002; Harmsen *et al.,* 2010). Surface of spider silk is also coated with several antibacterial polyunsaturated fatty acids such as 12-methyltetradecanoic acid that also generate inappropriate environment for the attachment of Gram negative bacteria (Sharma, 2014).

Spider silk prevent the formation of biofilm in Gram negative bacteria (Sharma, 2014). Biofilms are self-produced extracellular polymeric matrix in which bacterial colony is embedded (Mohammed *et al.,* 2013). Bacterial strains become resistant to antibacterial drugs by the formation of biofilms. Bacteria embedded in biofilms are protected from the host immune system and antibacterial substances because biofilms form protective matrices that prevent them from penetration into the cell (Stromstedt *et al.,* 2014). Naturally derived antibacterial products possess vast chemical diversity that prevent the formation of biofilms by bacterial colonies (Francolini *et al.,* 2004). Spider silk contain a non-protein amino acid called GABA (Gamma-amino-butyric acid) that resist the attachment and biofilm induction in Gram negative bacteria (Sharma, 2014). GABA is a four carbon amino acid commonly present in the environment (Bouché *et al.,* 2003).

Results of present study clearly indicate that spider silk is more effective against Gram negative bacteria compared to the Gram positive ones. This is contradictory to the findings of Wright and Goodacre (2012), who reported that spider silk is more potent against "*Bacillus subtilis"* a Gram positive bacteria and shows no effect on "*Escherichia coli"* a Gram negative bacteria. Roozbahani *et al.* (2014) also investigated the effect of silk obtained from *Pholcus phalangioides* against Gram negative (*E. coli*) and Gram positive (*L. monocytogenes)* bacteria. Silk of *Pholcus phalangioides* was found to be more effective against Gram positive bacteria than Gram negative bacteria. Bacteriostatic activity of spider silk varies with the type and quality of silk recovered from different species of spiders (Wright and Goodacre, 2012). Silk recovered from different species of spiders may differ in composition and arrangement of their amino acids in silk proteins. Biochemical study revealed that different types of silk hold at least two to three distinguished structural proteins, commonly known as fibroins (Hisa *et al.,* 2011). Furthermore, bacteriostatic activity of selective may also vary against different bacterial strains.

Two main mechanisms are followed by antibacterial drugs to affect the microbial cells. These drugs either adopt bacteriostatic or bactericidal mechanism. Bactericidal drugs kill the bacteria and bacteriostatic drug repress the growth of bacteria (Bernatova *et al.,* 2013). Wright and Goodacre (2012) and Sharma (2014) also reported in their studies that silk of spider operates in bacteriostatic manner to inhibit the growth of bacteria.

It is concluded although silk of *E. excelsa* is effective against both Gram negative "*Acinetobacter* sp." and Gram positive "*Streptococcus* sp." bacteria but silk of this species is more potent against *Acinetobacter* sp." More studies are needed to reveal the antibacterial components of silk of this species so that in future these antibacterial components can be synthesized and used in the formation of new antibiotics and other therapeutic products to treat the infectious diseases especially caused by the Gram negative bacteria.

Conflict of interest: Authors declare that they have no conflict of interest.

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