Short Communication

ACTA ZOOLOGICA BULGARICA
Acta zool. bulg., 69 (4), 2017: 593-595

Evidence for the Antimicrobial Potential of Silk of *Cyclosa confraga* (Thorell, 1892) (Araneae: Araneidae)

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Abstract: We evaluated the anti-bacterial potential of silk recovered from *Cyclosa confraga* (Thorell, 1892). The silk inhibited the growth of *Streptococcus* sp. and *Acinetobactor* sp.. Significant differences in the zones of inhibition among treatments were observed for both bacterial strains. The zones of inhibition produced by silk against *Acinetobactor* sp. (gram negative bacteria) were larger in size than the inhibition zones observed against *Streptococcus* sp. (gram positive bacteria) at all tested concentrations.

Key words: Spiders, silk, bacteria, anti-bacterial potential

Introduction

Microbial diseases are still a main threat to human health. Relatively, developing countries are more affected due to non-availability of medicines and resistance to antibiotics (Okeke et al. 2005). One of the causes of bacterial resistance is excessive and improper use of antibiotics for non-bacterial diseases (Levy & Marshall 2004). Thus, there is an urgent need to discover new antimicrobial compounds. There are varieties of natural sources that contain antimicrobial agents which could be used for the control of bacterial infections (Zuridah et al. 2008, Saleem et al. 2010).

Plants are the major source of natural antimicrobials (Gyawali & Ibrahim 2012). Animals are also a source of different antimicrobial substances (Hoskin & Ramamoorthy 2008). The silk of some spider species possesses antibacterial activity (Simon & Goodacre 2012). Spider silk has acidic properties which inhibit the growth of microbes. This indicates that it may have bacteriostatic effects (Saleem et al. 2010). Further, potassium nitrate is present in silk and inhibits the growth of microbes on silk (Chakraborty et al. 2009, Gomes et al. 2010). The antimicrobial potential of spider silk has been previously reported by various researchers worldwide (Saravanan 2006, Saleem et al. 2010, Mirghani et al. 2012, Amaley et al. 2014, Sharma 2014). Although spider silk has remarkable properties, it is least studied for its antibacterial potential. There are no published data on the antibacterial potential of silk from spiders occurring in Pakistan.

Materials and Methods

The present study was designed to evaluate in laboratory conditions the antibacterial potential of silk of *Cyclosa confraga* against two bacterial strains, *Streptococcus* sp. and *Acinetobactor* sp. *Cyclosa confraga* is an orb-web spider that is common in citrus orchards of the Sargodha and Punjab Districts, Pakistan. We hypothesised that silk of this spider species contain antimicrobial agents that could be used to inhibit the growth of a wide range of bacteria.

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Table 1. Comparison of inhibition zones (mm) appeared when 20 ul of three different concentrations of spider silk solution were applied against *Acinetobactor* sp. and *Streptococcus* sp.

Conc. of silk of spider	*Acinetobactor* sp.	*Streptococcus* sp.
100%	$24.33^{c} \pm 0.88$	$19.33^{c} \pm 0.66$
75%	$19.66^{b} \pm 0.88$	$18.00^{bc} \pm 0.57$
50%	$17.66^{b} \pm 0.33$	$15.33^{b} \pm 1.20$
Control	$0.41^{a} \pm 0.14$	$0.59^{a} \pm 0.19$
DF	*3,9*	*3,9*
F	*106.97*	*20.98*
P-value	*<0.001*	*<0.001*

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

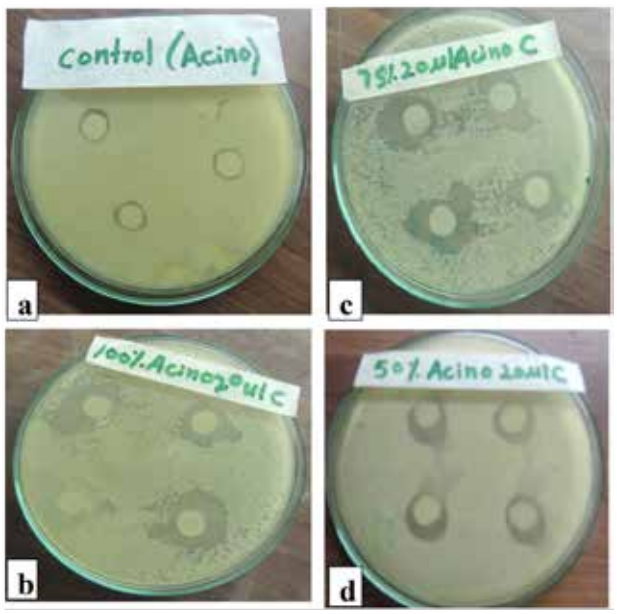

Fig. 1. Zone of inhibition against *Acinetobacter* sp. produced by various concentrations of spider silk solution.

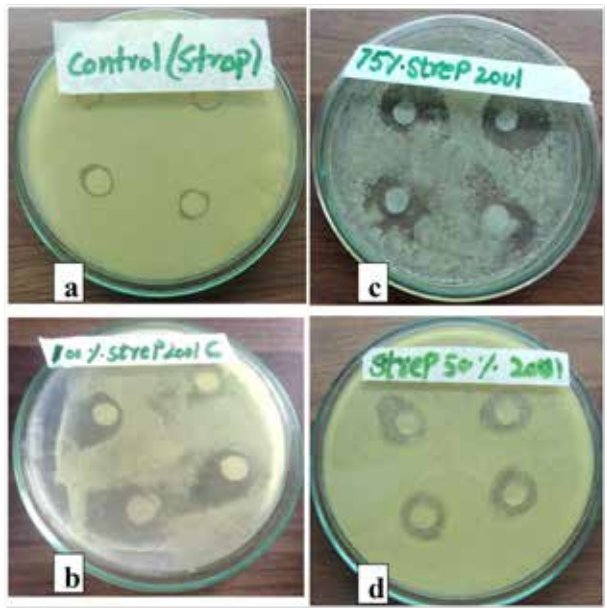

Fig. 2. Zone of inhibition against *Streptococcus* sp. produced by various concentrations of spider silk solution.

Hand picking was used for the collection of spiders. Specially designed wooden boxes were used to rear spiders in the laboratory for silk recovery. Spiders were offered live flies on daily basis. Humidity of 60-70% was maintained in the spider rearing room. In order to prepare stock solution, silk was collected from spider webs by a sterile glass rod and 50 mg were dissolved in 100 ml 2.5% sodium hydroxide solution. This stalk solution was used to prepare silk solutions of different concentrations (i.e., 100%, 75% and 50%); NaOH solution without spider silk was used for control treatments.

Two bacterial strains [one gram positive (i.e., *Streptococcus* sp.) and one gram negative (*Acinetobactor* sp.)] were used as test organisms. Both bacterial strains were borrowed from the University of Punjab, Lahore and cultured in the laboratory. Disc diffusion method was used to evaluate the antibacterial potential of silk from *Cyclosa confraga*. Both bacterial strains were treated with 20 µl of each concentration. Data was analysed statistically using SPSS (version 16). Turkey's test was used for multiple comparisons.

Results

Silk of *C. confraga* inhibited the growth of both *Streptococcus* sp. and *Acinetobactor* sp.. Clear zones of inhibition for these bacterial strains were observed at 24 hours post treatment (Figs. 1, 2). There were significant differences in the zones of inhibition among treatments ($F_{3,9}$=106.97; P<0.001 for *Acinetobactor* sp. and $F_{3,9}$=20.98; P<0.00 for *Streptococcus* sp.). Results of Tukey's test showed that zones of inhibition with 100% silk solution were larger in size as compared to the inhibition zones observed with 75%

and 50% silk solutions (Table 1). It was also evident that the zones of inhibition against *Acinetobactor* sp. (gram negative bacteria) were larger in size than the inhibition zones observed against *Streptococcus* sp. (gram positive bacteria) at all tested concentrations (Table 1).

Discussion

Previously, many researchers have reported the antimicrobial potential of spider silk (Saravanan 2006, Saleem et al. 2010, Amaley et al. 2014, Sharma 2014). Amaley et al. (2014) reported that the dragline silk of *Nephila pilipes* inhibited the growth of *Escherichia coli* (gram negative), *Staphylococcus aureus* (grams positive) and *Pseudomonas aeruginosa* (gram negative). They also recorded greater effect of spider silk on gram negative bacteria. Sharma (2014) also recorded that *Argiope aurantia* silk showed reduced bacterial attachment for gram negative bacteria on its surface.

Out results contradict the ones of Simon & Goodacre (2012). They have found that silk of the common house spider *Tegenaria domestica* is effective in inhibiting the growth of the gram-positive *Bacillus subtilis* but not against the gram-negative *Esherichia coli*. Similarly, Roozbahani et al. (2014) also reported antimicrobial activity of *Pholcus phalangioides* silk against gram positive bacteria, *Listeria monocytogenes.* The properties and composition of silk vary between and even within spider species (Vollrath & Knight 2001, Garrido 2002). Therefore, variation in responses to silk belonging to different spider species is expected.

Amino acid composition is also different in different types of silk (Work & Young 1987, Craig 1997). The difference in inhibitory effects of spider silk on gram negative and gram positive bacteria could also be possibly explained by the differences in cell wall structures of gram negative and gram positive bacteria (Donlan 2002, Harmsen et al. 2010). Therefore, although the silk of *C. confraga* inhibited growth of both types of bacteria, it was more effective in inhibiting the growth of gram-negative bacteria.

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Received: 28.09.2016
Accepted: 10.01.2017