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INVESTIGATION OF THE *PHOLCUS PHALANGIOIDES* SPIDER WEB FOR ANTI BACTERIAL ACTIVITY

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ABSTRACT

Spiders are one of the most successful predators, they are equipped with natures wondrous material known as spider silk. Spider silk extracts were prepared by using the solvent Dimethyl sulfoxide. The present study was based on the anti-bacterial properties of the *Pholcus phalengioides* commonly known as cellar spider web. For the assessment of its inhibitory activity four pathogenic bacterial strains *Staphalococcus aureus, Bacillus subtilis, Pseudomonas aeuroginosa* and *Escherichia coli* were used, where standard Gentamycin (80 mcg) was kept as positive control. Antibacterial activity was evaluated by Muller Hinton agar well diffusion method. Two different concentrations (400 mcg) and (800 mcg) of web extracts were used for the experiment. Spider web showed antibacterial activity against four bacterial strains. The spider silk induced the highest inhibition zone against *Bacillus subtilis,* followed by *Staphylococcus aureus* and *Psuedomonas aeruginosa.* The silk showed least effectiveness against *Escherchia coli.* Moreover, the inhibitory potential of silk varied with the concentrations of the silk solution. This study provides clues to the role of spider web extract in treating the pathogens effect of different microbes.

KEYWORDS: Spider silk, *Pholcus phalengioides*, anti-bacterial properties, inhibition zone, bacteria - *Staphalococcus aureus, Bacillus subtilis, Pseudomonas aeuroginosa* and *Escherichia coli,).*

INTRODUCTION

Spiders are one of the most successful predators of pest and pest populations (Agnarsson *et al.*,2010). At present, spiders constitute a diverse group of 114 families, 3935 genera's and 44,906 species occurring worldwide. Spiders play very essential part in our ecosystem and food chain. They are the major predators that naturally regulate and limit the pest populations of different crops (Mishra *et al.,* 2012). Each spider species produce different kind of web in accordance with the habitat life style and food availability. At a time more than one kind of silk can be produced by the spiders (Foelix 1996).

Today, the medical and pharmaceutical industries are trying to develop an effective treatment against the infectious diseases. In this aspect the antimicrobials derived from natural resources is very useful to produce the effective and bio-friendly therapeutic substances. Thus in this present age of alarming health concerns the spider silks could prove miraculous substances having potent antimicrobial activity. The lipids present in spider silk contain 12Methyl tetradecanonic acid and 14-Methyl hexadecanonic acid and that inhibit growth of microbes.

The chemicals present in the spider silk are very useful to present themselves from the enemies and also they can preserve the extra food for their later use and also this reserve food is free from bacterial and fungal attack. Antifungal and antimicrobial compound such as bisphosphonates peptides, phospholipids hydrate and potassium nitrate is also observed in silk fibers (Chakarabarty and Das, 2009 and Gomes *et al.,* 2010). Applications of spider silk in medical field and life-sciences is increasing nowadays (Altman *et al.,* 2003 and Bourzac, 2015).

Spider silk possess outstanding and valuable therapeutic, wound healing and regenerative properties (Shear *et al.,* 1989; Seenivasan *et al.,* 2005). Mahdi *et al.,* (2014), studied the antimicrobial properties of spider silk *Pholcus phalangioides* produced in sterile conditions against two bacteria, *Listeria monocytogenes* and *Escherichia coli* with using well diffusion method and Macro Broth Dilution method was evaluated. The results showed that the antimicrobial compounds present in the solution spider silk greater inhibitory effect on gram-positive bacteria *L. monocytogenes* than Gram-negative bacteria *E. coli.* A study conducted by Shahbuddin *et al.*, (2016) explained Spider silk contains peptides and biomolecules that able to stimulate and improve conditions of wound healing. The present study was specifically designed to evaluate the antibacterial potential of spider silk against standard drug resistant

pathogenic bacteria. This study also aims to understand the non-diffusible antibacterial compound present in the spider silk.

MATERIALS AND METHOD

Collection of Spider Web

Spider web are collected from rooms inside the house; it is mainly collected from Palakkad District of Kerala.

Preparation of Web Extracts

Spider web is washed with distilled water. After that it is transfer in to the test tube, and shake it continuously by using the solvent DMSO. Solvent is evaporated to get crude extract and these extract is used for antibacterial test.

Antibacterial Assay By Agar Well Diffusion Method Procedure

Agar well diffusion method is widely used to evaluate the antimicrobial activity of the plant extracts. Same amount 15-20 mL of Mueller-Hinton agar was poured on glass Petri plates of same size and allowed to solidify. Wells with a diameter of 8 mm (20 mm apart from one another) were punched aseptically with a sterile cork borer in each plate. Standardized inoculums of the test organism were uniformly spread on the surface of these plates using sterile cotton swab. A volume (50 µL) of the extract solution at desired concentration was added to the wells and one well with Gentamycin maintained as positive and DMSO as a negative control. Then, the agar plates were incubated under suitable conditions depending upon the test microorganism. After incubation, clear zone was observed. Inhibition of the bacterial growth was measured in mm.

Culture medium details

Mueller Hinton Agar M173 HiMedia used for determination of susceptibility of microorganisms to antimicrobial agents. Suspend 38 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Inoculums details

Inoculums were procured from The Microbial Type Culture Collection and Gene Bank (MTCC) Chandigarh.

Bacteria	MTTCC No	Incubation condition
Staphylococcus aureus	87	37^0C for 24 hours
Escherichia coli	443	37^0C for 24 hours
Bacillus subtils	2413	37^0C for 24 hours
Pseudomonas aeruginosa	424	37^0C for 24 hours

RESULT

The antimicrobial potential of spider web extract was assayed on four bacterial strains. The antibacterial activity was evaluated by well diffusion agar method and the diameter around the inhibition zone was measured by using (mm) scale. The extracts showed selective antibacterial activities along the four strains. When the bacterial growth tested on the range of two different dosage greater inhibition was observed in higher dosage.

The present study was focused on the bacterial activity of spider web extract by using the solvent DMSO. The extracts were subjected to antimicrobial activity against gram positive (*S.aureus*, *B.subtils*) and gram negative (*P.auerginosa* and *E.coli)* bacteria.. The results are listed and showed in **(Table 1-3).**

Spider web from the kitchen area (fuel smoke area) showed potent antimicrobial activity against the gram positive and gram negative bacteria.

In the sample which was collected from kitchen area showed different zone of inhibition. When we observed gram positive bacteria *B. subtils* showed 15 mm and 24 mm inhibition zones at T1 (400 mcg) and T2 (800 mcg) concentrations; *S. aureus* showed 14 mm and 20 mm inhibition zones at T1 and T2 concentrations. But in the case of gram negative bacteria *P. auerginosa* showed 15 mm and 23 mm inhibition zone at T1(400 mcg) and T2(800 mcg) concentrations; *E. coli* showed 12 mm and 20 mm inhibition zones at T1 and T2 concentrations.

The maximum zone of inhibition was observed gram positive *B. subtils* (24mm) at 800 mcg in T2 concentration; gram negative *P. auerginosa* showed (23 mm) zone of inhibition at 800 mcg. This value was compared with standard gentamycin, showed higher inhibition zone (29 mm) than the spider web extracts. The maximum zone of inhibition at 400 mcg in T1 concentration is (15 mm) in *P. auerginosa* and the lowest zone of inhibition at 400 mcg is (12 mm) in *E. coli.*

Table 1: Antibacterial Assay – Gram Positive Bacteria.

Organism name		*Staphylococcus aureus*	*Bacillus subtilis*
Samples	**Concentration of samples**	**Zone of**	**Inhibition(mm)**
CSV II	Standard Gentamycin (80mcg)	20	29
	Negative control	-	-
	T1 (400mcg)	14	15
	T2(800mcg)	20	24

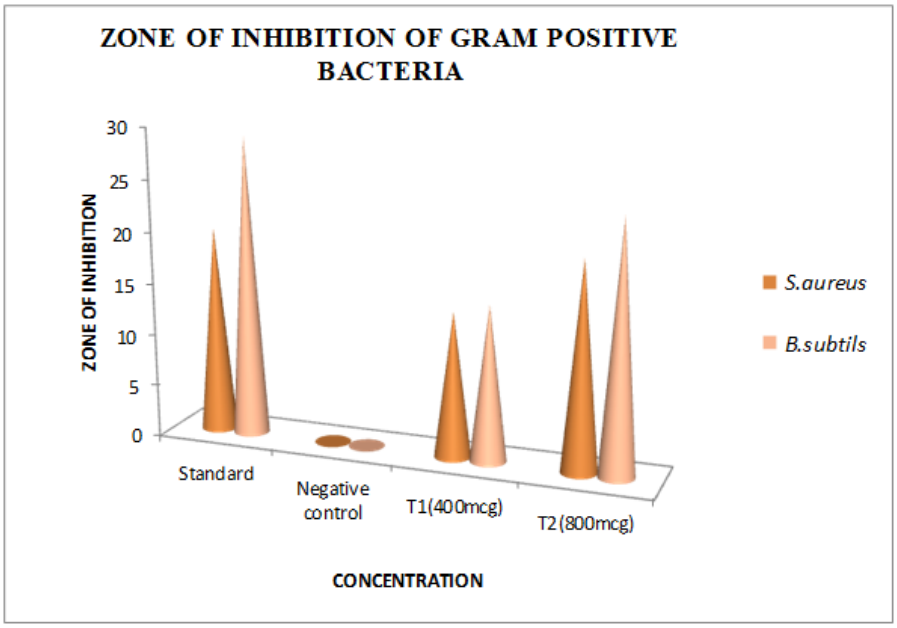


Table 2: Antibacterial assay – Gram Negative Bacteria.

Organism name		*Escherichia coli*	*Pseudomonas aeruginosa*
Samples	**Concentration of samples**	**Zone of inhibition(mm)**	
CSV II	Standard Gentamycin (80mcg)	24	28
	Negative control	-	-
	T1 (400mcg)	12	15
	T2(800mcg)	20	23

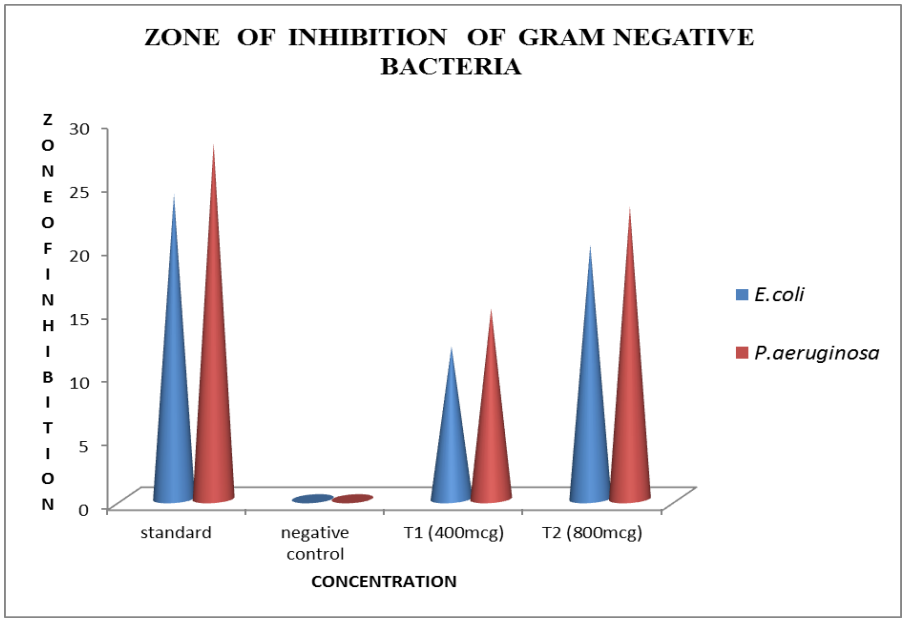
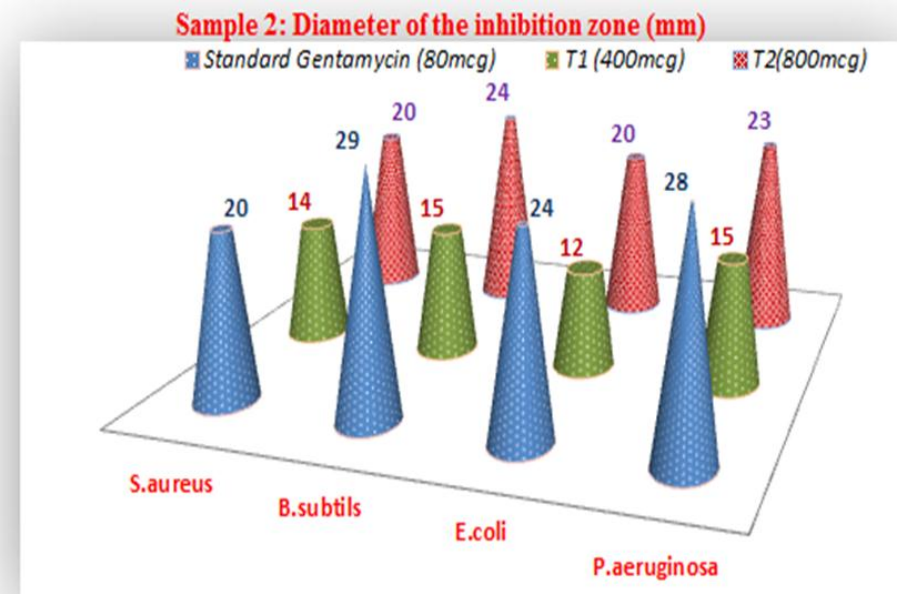


Table 3: Sample (Web collected from kitchen area).

Concentration of sample		Diameter of the inhibition zone (mm)		
	S.aureus	***B.subtils***	***E.coli***	***P.aeruginosa***
Standard Gentamycin (80 mcg)	**20**	**29**	**24**	**28**
Negative control	**-**	**-**	**-**	**-**
T1 (400 mcg)	**14**	**15**	**12**	**15**
T2(800 mcg)	**20**	**24**	**20**	**23**



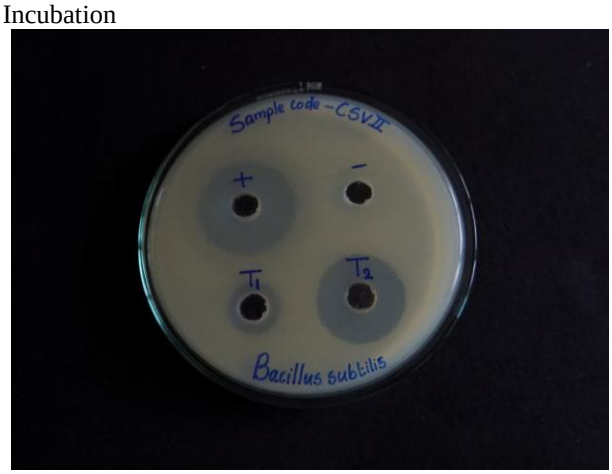
MEAN VALUE AND STANDARD DEVIATION OF THE SAMPLE

Sample (Spider web from kitchen/ smoke rich area)						
Concentration of sample	**Diameter of the inhibition zone (mm)**					
	S.aureus	***B.subtils***	***E.coli***	***P.aeruginosa***	**x̄**	**σ**
Standard Gentamycin (80 mcg)	20	29	24	28	25.25	2.09
Negative control	-	-	-	-	--	---
T1 (400 mcg)	14	15	12	15	14.00	2.44
T2(800 mcg)	20	24	20	23	21.75	3.56

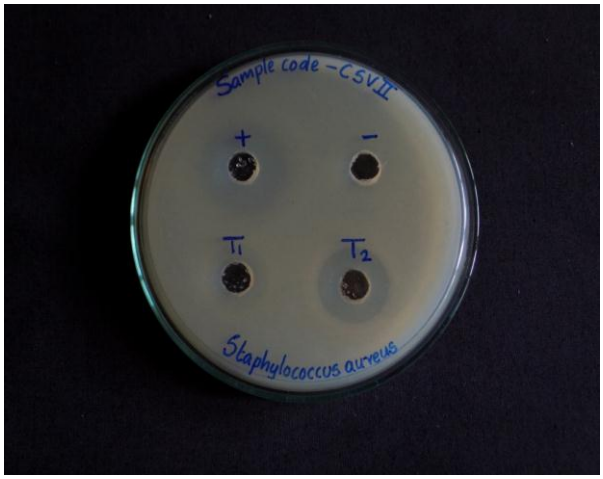
x̄ = Mean Value, σ = Standard deviation

Plates Showing The Inhibition Zones At Different Concentration

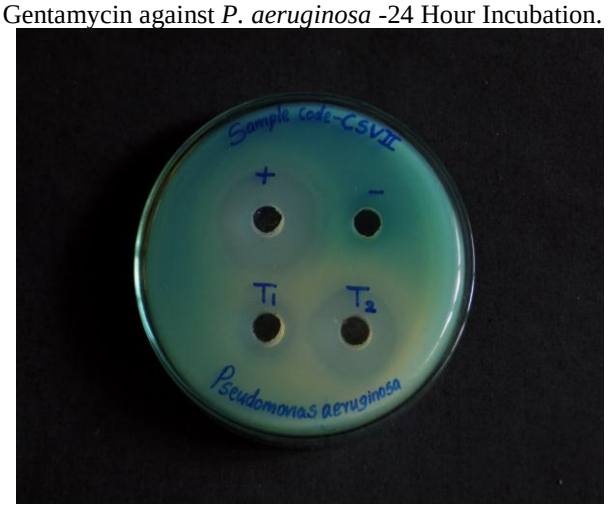
Antibacterial activity of spider extract 400mcg, 800 mcg and
Antibiotic Gentamycin against *B. subtils*- 24 Hour Incubation



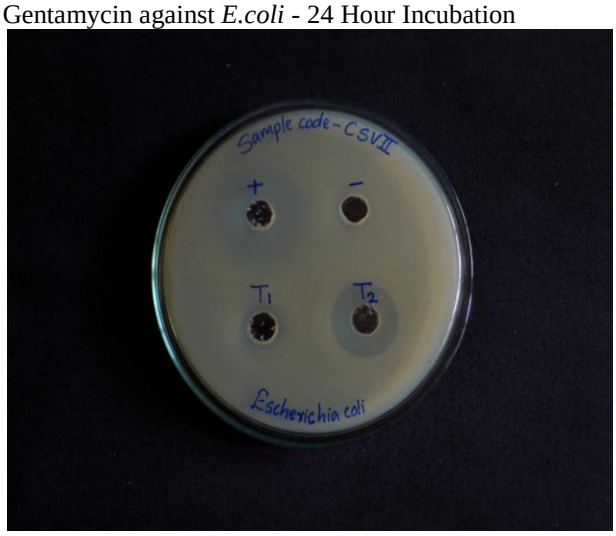
Antibacterial activity of spider extract 400 mcg, 800 mcg and
Antibiotic Gentamycin against *S. aureus* - 24 Hour Incubation



Antibacterial activity of spider extract 400mcg, 800mcg and Antibiotic
Gentamycin against *P. aeruginosa* -24 Hour Incubation.



Antibacterial activity of spider extract 400 mcg, 800 mcg and Antibiotic
Gentamycin against *E.coli* - 24 Hour Incubation



DISCUSSION

The present investigation was successfully completed with the collection and culture of *P. phalengioides* spider species. Our results revealed the significant inhibitory effect of cellar spider against *B. subtils*, *E. coli*, *S. aureus*, and *P. aeruginosa*. Many researches have discussed the spiders silk inhibit growth of in (Fairbrothere *et al.*,1997). Antibacterial effect of spider silk against both gram positive and gram negative strain have also been reported by Chakrabaty and Das (2004).

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 (Gomes *et al*., 2010).

The present study, antimicrobial activity of spider web was investigated by using some species of bacteria and. All the four bacterial isolates are gram positive *S. aureus, B. subtils* and gram negative *E. coli, P. auerginosa* suppressed by spider web extract. Here the explanation given by (ZafarIqbal *et al.,*2019) is valid. Spider web have evolved specific antibacterial activity against the bacteria that are potential to them. The present study spider species, *P. phalengioides* spider web extract showed a strong significant antimicrobial activity (Hafiz Tahir, 2018).

Anti-microbial compounds such as bisphosphonates peptides, phospholipids hydrate and potassium nitrate were also observed in silk fibres (Chakraborty and Das, 2009 and Gomes *et al.,* 2010). Spiders silk is contains amino acids including glycine and alanine and large amounts of pyrrolidine helping to keeps the water in the spider silk and will protect it from drying out. In addition, phospholipids hydrate and potassium nitrate available at spider silk can prevent from the growth of fungi and bacteria on the silk (Chakraborty *et al*., 2009; Gomes *et al*., 2010).

Our study also reveals that the spider silk is potently more inhibitive for the growth of Gram positive bacterial strains as compared to Gram negative ones. We found that the largest zones of inhibitions were recorded in *B. subtilis* followed by *P. aeurginosa, S. aureus* and *E. coli* bacterial strains. Our studies are closely related to the findings of Wright and Good care (2012), 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.

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 and Goodacre (2012) indicated that only *B. subtilis* is susceptible to the inhibitions induced by spider silk.

CONCLUSION

This study demonstrates that spider web (*Pholcidae* species) extract have anti-bacterial properties. This study provides clues to the role of spider web extract in treating the pathogen effect of different microbes. According to the result, can be concluded that extract of spider web can be used to formulate a new natural antimicrobial product for controlling infections of

multidrug resistant bacteria where treatment is very difficult as the drug of choice for treating infection doesn`t work. This study may thus lead to formulation of new natural antimicrobial agent and thus may be beneficial in future prospects for mankind.

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