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SPIDER WEB OF *PHOLCUS* SP.- A POTENTIAL NATURAL PRODUCT AGAINST BACTERIAL PATHOGEN

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ABSTRACT

In recent years much attention is focused on ethno zoology. Many traditional healers have used animal products to cure many ailments. The traditional healers have used the spider web and other insect products to treat chronic diseases, wound, skin diseases etc. With this background in the present study the medicinal properties of the products of spider web was studied. The human pathogenic microbes responsible for many infectious diseases were tested for their growth inhibitory activity using the extracts of web of the spider. In the present study 8 bacterial isolates *V.vulnifiucus, P.flurescence, S.aureus, E.coli, S.mutans, E.faecalis, S.marcesens,* and *Methicilin resistant Staphylococcus aureus* were tested against the extract of spider for antimicrobial activity. The spider web extract showed a good inhibitory activity against *S.aureus* and *S.mutans.* and *E.coli* From this study it is clear that the bio active compounds and the protein profile of the extracts induce antimicrobial activity.

KEYWORDS: Spider extract; antimicrobial; spider web; pathogen; infection.

INTRODUCTION

Spiders evolved about 374-380 million years ago and at present, spiders constitute a diverse group of 114 families, 3953 genera and 4, 49,906 species occurring in the world. Spiders spin web and the material in the web is believed to have medicinal properties and this has been used by traditional healers to cure chronic wounds. The antimicrobial properties of the silk of the spider *phalangioides* was tested against two bacteria, *Listeria monocytogenes and Esherichia coli (*Roozbahani et al., 2014). Applications of spider silk in medical field and

life-sciences is increasing nowadays (Altman et al., 2003; Bourzac, 2015). Spider silk possess outstanding and valuable therapeutic, wound healing and regenerative properties (Shear et al., 1989; Seenivasan et al., 2005). This makes spider silk a remarkable and extraordinary biomaterial (Gosline et al., 1999; Vollrath, 2000). Spider silk play important role in the regeneration of many tissues and body cells such as skin, nerve, bone, and cartilage (Altman et al., 2003; Schneider et al., 2009; Sinokowska, 2011; Wendt et al., 2011). Many damaged connective tissues such as tendons and ligaments can also be repaired (Schneider et al., 2009; Sionkowska, 2013; Bourzac, 2015). Spider silk films supply structural support and induces regeneration in the tissues (Sinokowska, 2011). Silk fiber can also be used to treat nerve disorders and the silk fibres showed biocompatibility and very low immunogenicity when grown with The spider silk fibres were reported to enhance healing of injury because it improves the regeneration of damaged cells. Hence an attempt has been made to evaluate the biomedical potential of the bioactive compounds present in the fibre of the web of the household spider *Pholcus* sp.

2. MATERIALS AND METHODS

Spider web Silk collection

The Spider web of the common house spider *Pholcus sp.*, was used for the present study. Spider web silk was collected from household locations and hostel building using a stick. It was then transferred into a sturdy plastic bag and closed tightly. It was weighed using an electronic balance.

Selection of Bacteria for the Study

Multiple antibiotic resistant isolates, which include *V.vulnifucus, P.flurescence, S.aureus, E.coli, S.mutans, E.faecalis, Methicilin resistant Staphylococcus aureus* and *S.marcesens* which are cultured in the research laboratory, Animal Science Dept., were used for the present study.

Preparation of the Spider web extract

Spider web weighing 10g was taken and the dust was removed and the web was sterilized by placing it in the UV chamber subjected it to UV radiation for 30 minutes. Acetone was used as the solvent. Web silk was mixed with acetone and it was grinded well using a mortar and pestle. After grinding the content of the paste was removed and it was dried by evaporating it in the Hot air oven for 1 day at a temperature of 50^{o}C. To this powder 200ml of 50% DMSO was added and mixed well and filtered. The filtrate was used as the stock solution. From the

fresh stock solution different concentrations such as 20%, 40%, 60%, 80% and 100% were prepared and used for the anti-microbial assay, as it is done for silk worm silk anti-microbial assay.

Antibacterial Potential of the silk of the web of spider *Pholcus sp.*

The web of the spider is a product of protein secreting glands. The protein present in the fibre is also having anti microbial properties as the protein rich cocoon of *B. mori*.

In the present study the antibacterial potency of the crude extracts of the web of the spider *Pholcus sp.*, was tested. Of the 8 bacterial strains tested viz *V.vulnifucus, S.marcesens, S.aureus, S.mutans, P.fluorescence, E.faecalis, Methicilin resistant staphylococcus aureus* and *E.coli, Methicilin resistant staphylococcus aureus* and *staphylococcus mutans* showed a maximum diameter in the zone of inhibition (16mm) at an exposure with 100% concentration of the extract. Next to these strains the higher inhibitory activity was observed for *Serratia marcescens, Staphylococcus aureus* and *E.coli* (15 mm) at 100% dose. For all the other bacteria the diameter of the zone of inhibition was 14 mm for 100% concentration of the extract. A concentration dependent inhibitory activity was observed. In the case of *E.coli* the sensitivity to 100% concentration of the extract is very near to the control (18mm). The diameter of the zone of inhibition for 40% and 60% extract was 11mm for *S.marcesens, S.aureus and P.fluorescence.* Likewise the zone of inhibition for 60% and 80% extract was 13mm for E.*faecalis*. (Table 1.; Fig. 1-12, Plate 1-3). From the present observation it is clear that the web of the spider has antimicrobial component.

DISCUSSION

The silk secreted by the spiders form a protective net around the spider. The protective net or the web trap the predators and help the spiders to capture the prey. The web of spider is composed of silk protein. Spider silk is characterized as fibrous protein having molecular weight greater than 200 KDa Vollrata (1999) and Altman et al., (2003). The silk protein is made up of non essential amino acids including Glycine and Alanine, Craig (1997). Craig & Rickel, (2002) reported the presence of the amino acid Arginine, serine Leucine, glutamine and tyrosine in the web silk.

The protein B – sheets present in the silk provide stretching ability of fibre Kluge et al., (2008). Spider silk is used as a bio material due to its high tensile strength, elasticity and extreme hardness (Tahir et al., 2017). Newman and Newman (1995) reported that the

prehistoric man used spider web to dress wounds. Altman et al., (2003) and Seenivasan et al., (2005) and Bourzac (2015) informed that the spider silk contains outstanding and valuable wound healing and regenerative properties in spider silk. Due to the high bio compatibility nature and the presence of antimicrobial agents the spider web can be used for different medical applications. Today spider web is used in the formation of artificial muscles, tendons and ligaments Macintosh et al., (2004) Carig et al., (1977) reported antimicrobial activity in spider web. The lipids present in the spider silk contain 12 / methyl tetra decanoic acid and 14 / methyl hexa decanoic acid that inhibit the growth of microbes Heimer (1988). Pohl et al., (2011) proved that the 12 / methyl tetra decanoic acid present in the spider web inhibited the growth of rice pathogens, Magnaprothe orizae. Heimer (1988), and Tahir et al., (2017) observed that the microorganisms are unable to grow on spider silk because of acidic nature and the spider web also contains two Bio active peptides SCP – 1 and SCP -2.

But in the present study the growth of ***E. coli*** was much inhibited by the extracts of the web of spider. As spiders are Arachnids organisms with several million years of life on earth should have developed some natural self-defensive mechanism through their secretions. Eberhard et al., (2006) found the food un decomposed or spoiled for several years when it is folded in silk fibres of the web. Antifungal and antimicrobial compounds basically induce growth inhibition zone in both gram positive and gram negative bacteria i.e., *Listeria monocytogenes* and E. coli Roozbanhani et al., (2014). In another study it was observed that gram positive bacteria are more susceptible than gram negative bacteria by spider silk when its growth inhibition effect was determined Mirghani et al., (2012). The role of spider silk to kill microorganisms was also reported by Charkraborty and Das (2009).

From the present study and from the previous reports it is quite evident that the spider web has a lot of medicinal applications. Although the spider silk has proven to be a miraculous substance having a wide range of applications yet its bio safety level has to be studied in detail.

Table 1: Showing the antibacterial activity of the extracts of web of the spider *Pholcus sp.*

Bacteria	Diameter of Inhibition Zone (mm)					
	Treatment Concentration %					
	20%	40%	60%	80%	100%	Control (Standard Antibiotic)
S. mutans	11	11	12	14	16	24
S.marcescens	9	11	11	13	15	20
V.vulnificus	11	11	12	14	14	23
S.aureus	10	11	11	13	15	22
MRSA	11	12	13	14	16	25
E.faecalis	10	11	13	13	14	24
E.coli	9	10	10	13	15	18
P.fluorescence	10	11	11	12	14	20

Plate 1: Image of spider, its web and treatment of fibre.

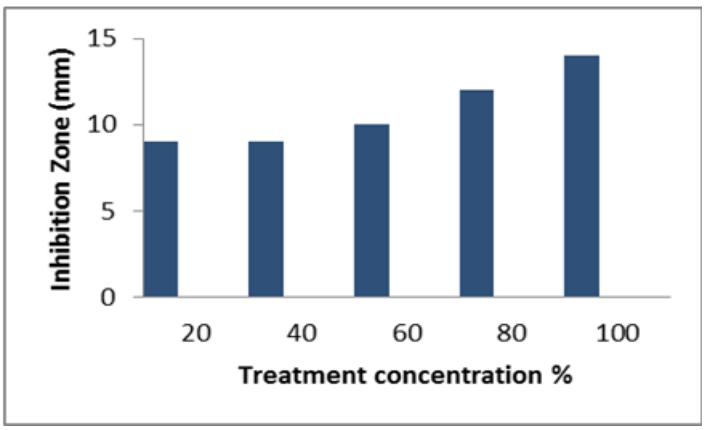

Fig. 1: The effect of the different concentrations of the spider web extract on the growth of the bacteria *S. mutans.*

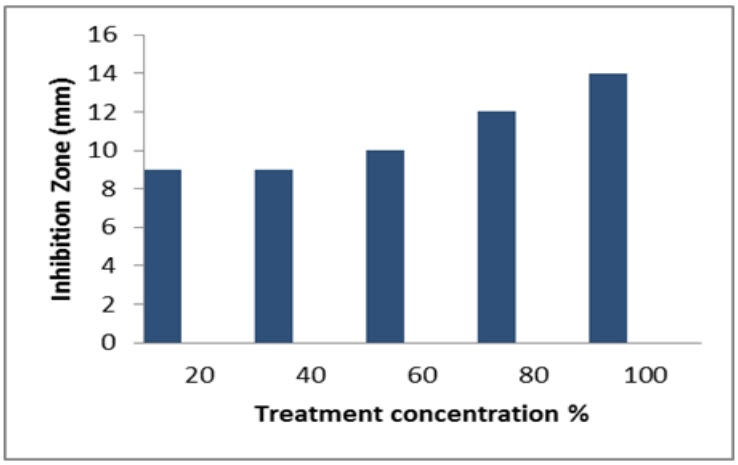

Fig. 2: The effect of the different concentrations of the spider web extract on the growth of the bacteria *S. marcescens.*

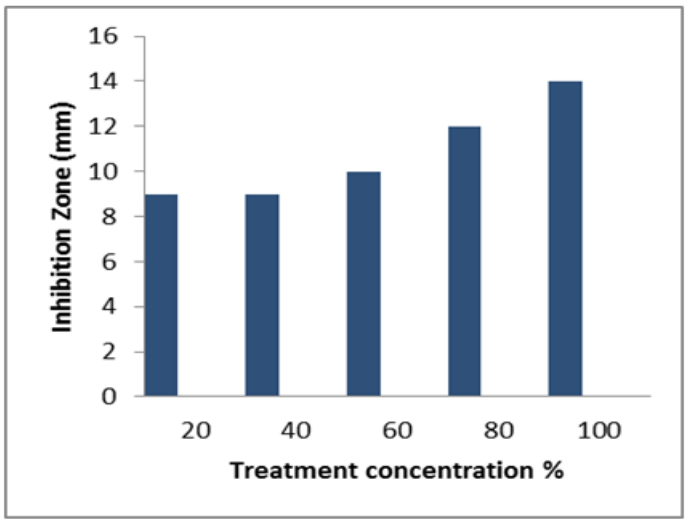

Fig. 3: The effect of the different concentrations of the cocoon extract on the growth of the bacteria *V. vulnificus.*

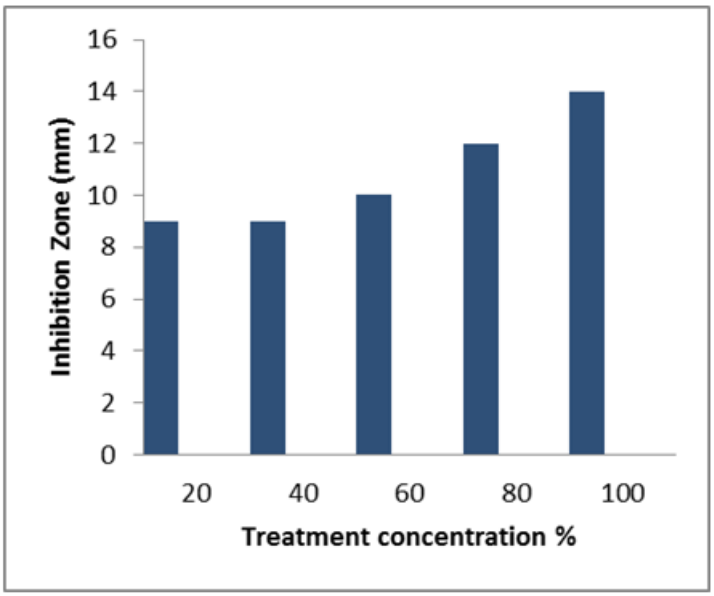

Fig. 4: The effect of the different concentrations of the spider web extract on the growth of the bacteria *S. aureus.*

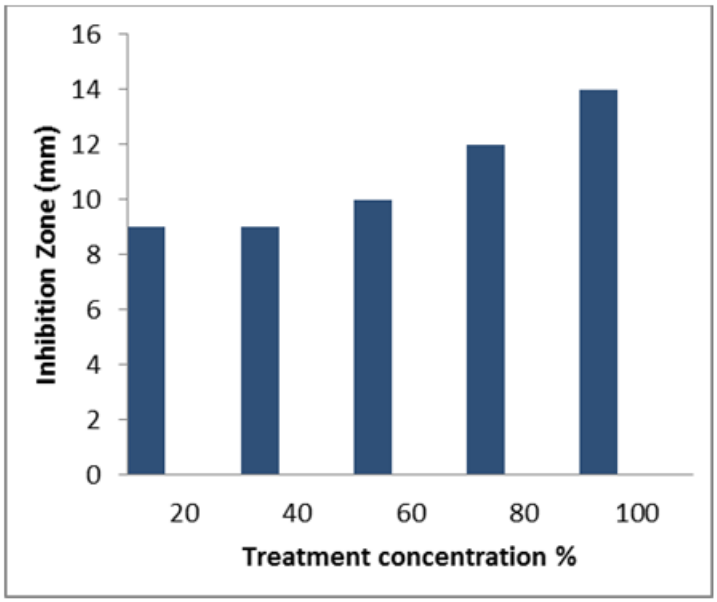

Fig. 5: The effect of the different concentrations of the spider web extract on the growth of the bacteria *Methicilin resistant Staphylococcus aureus.*

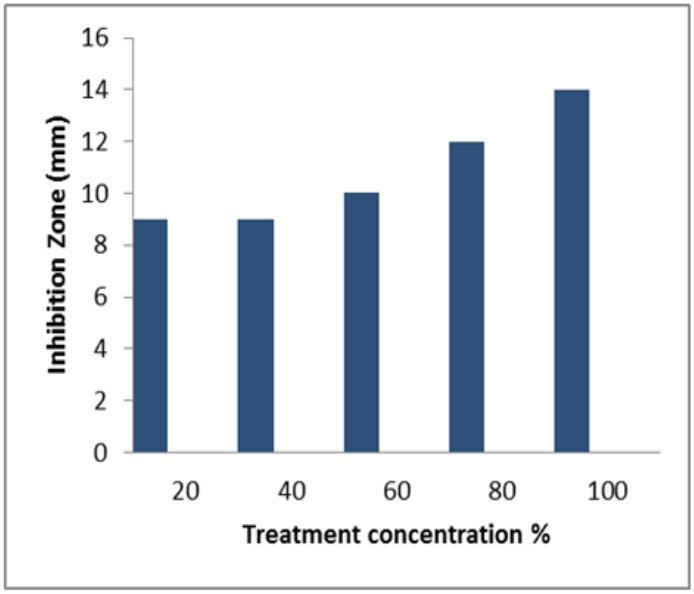

Fig. 6: The effect of the different concentrations of the spider web extract on the growth of the bacteria *E. faecalis.*

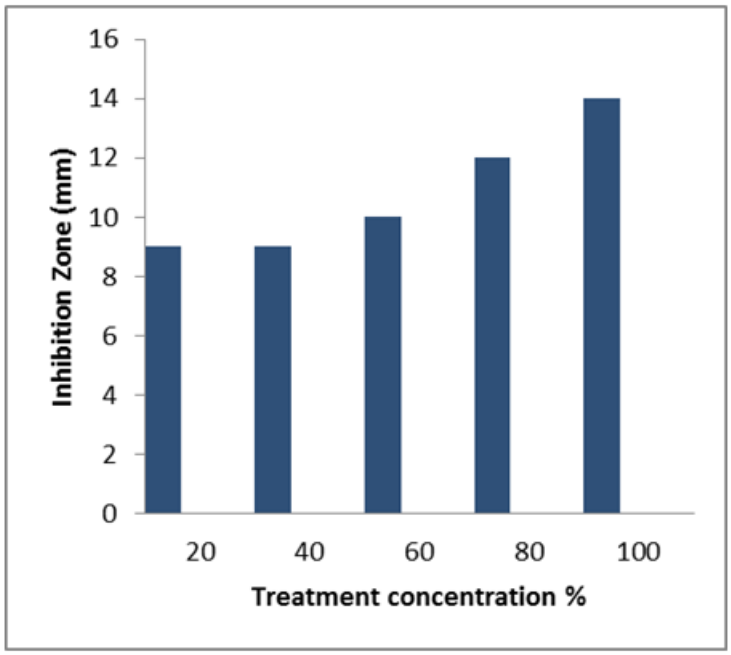

Fig. 7: The effect of the different concentrations of the spider web extract on the growth of the bacteria *E. coli.*

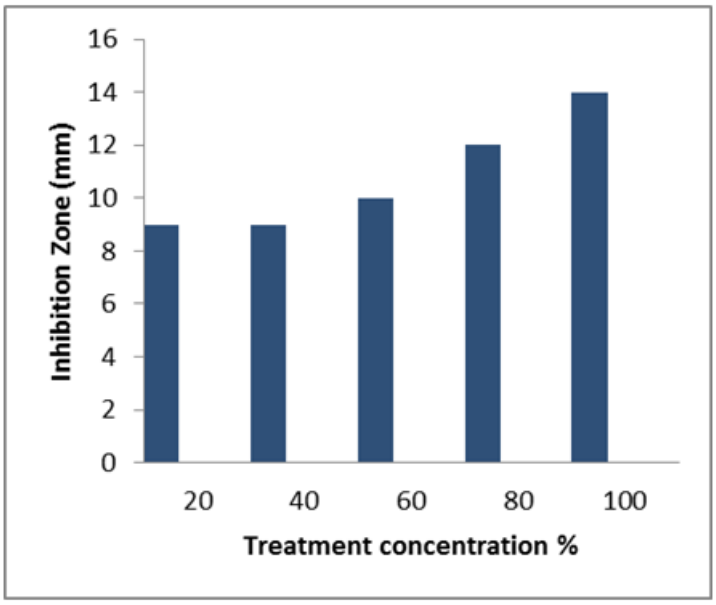

Fig. 8: The effect of the different concentrations of the spider web extract on the growth of the bacteria *P. fluorescence.*

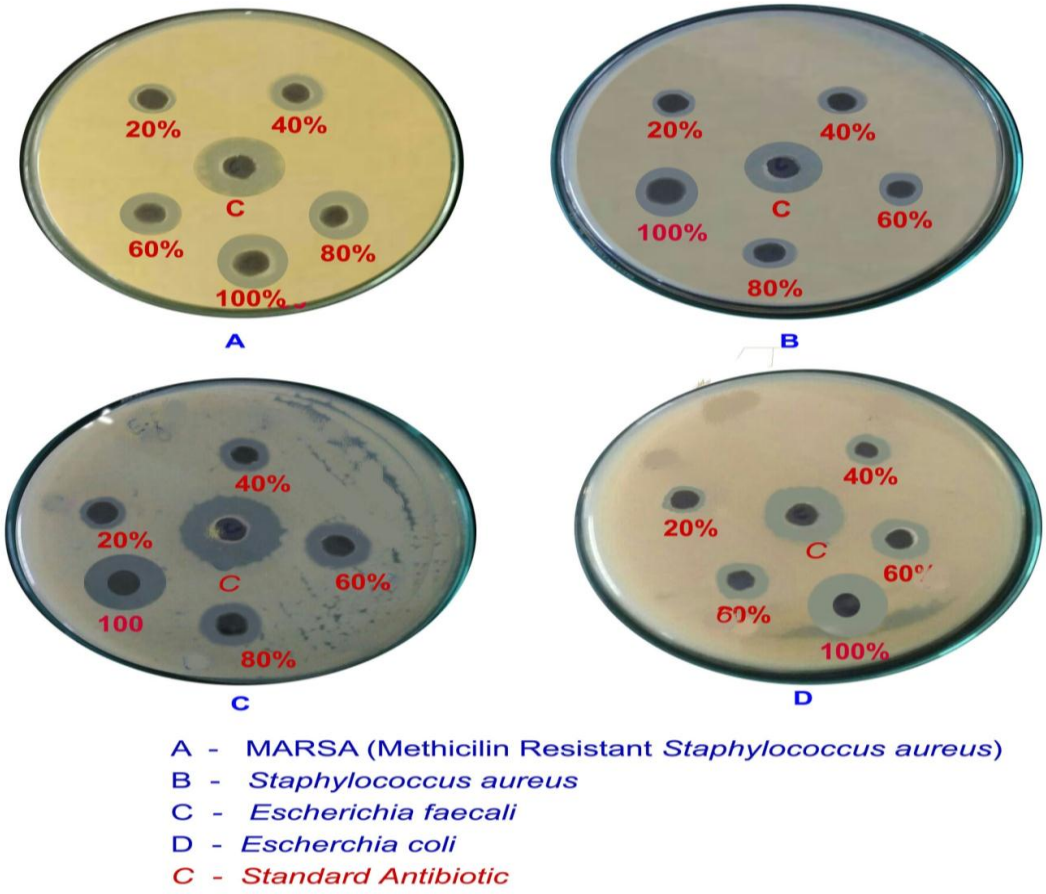

Plate 2: Antibacterial activity (Zone of inhibition) of the extracts of web silk of the spider Pholcus sp.

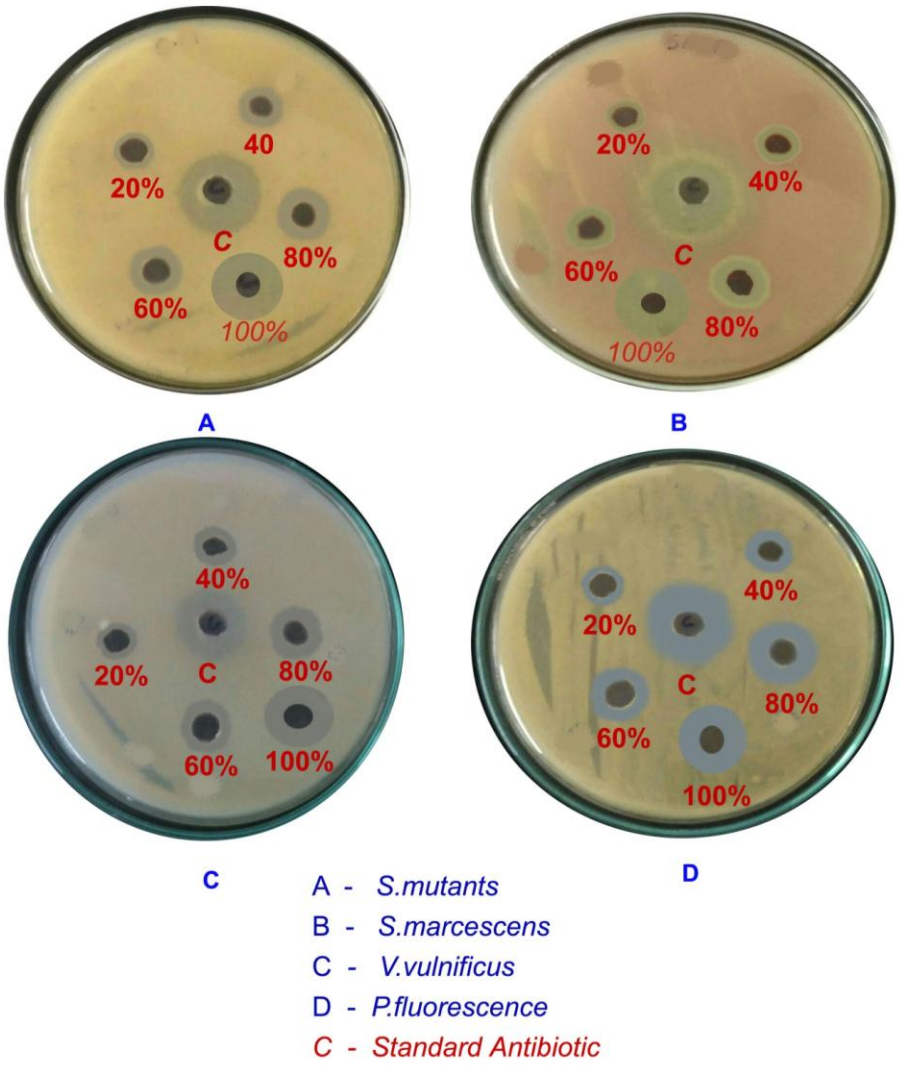

Plate 3: Antibacterial activity (Zone of inhibition) of the extracts of web silk of the spider *Pholcus* sp.

CONCLUSION

The arachnid spider secretes a highly protective webbed zone around itself so as to protect itself from predators. After the story of Robert Bruce the spider web has become a fascinating one. The fibre secreting gland in the caudal region of the spider produces a beautiful architectural protective zone. The traditional healers and tribes were known to apply the spider web for healing the wounds believing that it is having a wound healing property. Probably the can cure the wound after inhibiting pathogens. To confirm this ethno zoological practice the extracts of spider web was tested for antibacterial activity against gram positive and gram negative human pathogens. The Methicillin resistant Staphylococcus aureus showed a high sensitivity towards the extract of spider web. So it is undoubtedly clear that

the spider web contains antibacterial properties. This study will help to develop novel antibiotics from a natural source in the present context of growing antibiotic resistance.

FUTURE SCOPE

The study is expected to pay attention to ethno zoology.

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Conflict of Interest: NIL.

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