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A STUDY ON ANTIBACTERIAL ACTIVITY OF VENOM OBTAINED FROM NASTY RED FIRE ANTS (SOLENOPSIS SP.)

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ABSTRACT Ants are one of the most successful insect groups on the planet. They are often large, complex societies. Ants of the genus Solenopsis are known as fire ants because of their painful stings. The sting is used for colony defence, prey capture, and antimicrobial action. Fire ant venom is an aqueous solution. It contains proteins, venom alkaloids and is used against their enemies. An investigation was carried out in the laboratory to evaluate the antibacterial activity of the Nasty red fire ant venom extract. The antibacterial activity of nasty red fire ant extract was determined on selected bacterial strains. Well diffusion method of nasty red fire ant venom extract showed the antibacterial activity against the strains Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus Inhibition zone was absent in Enterococcus faecalis, Escherichia coli and Bacillus subtilis. In this study, the ant venom extract showed inhibitory effect on some common bacterial strains.

KEYWORDS : Formicidae, Hymenoptera, Solenopsis, Inhibition zone)

INTRODUCTION

Insects are highly specialized group of invertebrates belonging to the largest phyla, Arthopoda. Due to painful stings, ants belong to the genus *Solenopsis* is called fire ants (Qun-Hui Shi *et al.,* 2015). Many organisms use venom for attacking their prey and or for defence (Haight and Tschinkel, 2003). Venom of the genus *Solenopsis* is characterized by the presence of up to 95% water-insoluble alkaloids and a small amount of protein (Baer *et al.,* 1979; Jones *et al.,* 1982). The antibacterial properties of synthetic fire ant venom alkaloids were tested by both well diffusion and disc-diffusion procedures against a variety of bacteria (Jouvenaz *et al,,* 1972). Fire ant produces the venom in poison gland and stored in venom sac. It is delivered through the sting (Fox *et al.,* 2010). For the collection of fire ant venom alkaloids there are several techniques have been used. In this way, milligram quantities of pure venom can be obtained for direct use. Body soaking of living or dead ants for venom extraction is simple and effective. Even though whole body solvent-soak extraction is often used for fire ant venom alkaloid analysis (Hong-Wei Liu *et al.,* 2017). Ants in *Solenopsis* genus can cause severe impacts on human activities and the environment. Also cause medical problems. The worker ants sting and inject venom that can cause localized sterile blisters and whole body allergic reactions (Bastiaan Drees, 2014). When their nests are disturbed or attacked only they react aggressively (Fox, 2014). While most people can tolerate many stings (Bastiaan Drees, 2014). Hence the present study evaluating the antibacterial activity of nasty red fire ant venom on common human pathogenic bacteria.

MATERIALS AND METHODS

The ants were collected from Calicut district of Kerala and used for antibacterial activities.

SAMPLE PREPARATION

Take the weight of a clean glass beaker. Add 20 ml of hexane and transfer 30 to 40 live ants into the solvent. Then add 10 ml of distilled water. The extraction mixture separated into two phases. After a few minute, remove the dead ants using a clean forceps and evaporate the hexane-water mixture, then take the final weight of the beaker. Prepare stock solution using DMSO.

MICROBES SELECTED FOR STUDY

Gram positive bacteria (*Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis*) and Gram negative bacteria (*Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli*).

ANTIBACTERIAL ACTIVITY

Antibacterial activity was tested by agar well diffusion method. 15-20 ml of Muller Hinton agar was poured on glass petri plates and allowed to solidify. Wells with a diameter of 8mm (20 mm apart from one another) were punched aseptically with a sterile cork borer. 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, a clear zone was observed. Inhibition of the bacterial growth was measured in millimetre.

RESULT

ANTIBACTERIAL ASSAY

The antibacterial potential of the venom of nasty red fire ant was assayed on six bacterial strains. The venom extract showed selective antibacterial activities along the six strains. The venom extract were tested at two different concentration of 400 mcg and 800 mcg. The present study was focused on antibacterial activity of nasty red fire ant venom extract against both Gram positive (*Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis*) and Gram negative bacteria (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli*). The results are listed in table (Table - 2& 3).

The gram negative bacteria such as *Klebsiella pneumoniae* shows higher zone of inhibition (15 mm) at a concentration of 800 mcg among the six bacterial strains. *Pseudomonas aeruginosa* (14 mm) and *Staphylococcus aureus* (10 mm) showed inhibition zone at concentration of 800 mcg. Inhibition zone was absent in *Escherichia coli*, *Enterococcus faecalis* and *Bacillus subtilis*.

Table:1- Anti-Bacterial Assay against Gram Positive Bacteria

Name of organisms used	Staphylococcus aureus	Enterococcus faecalis	Bacillus subtilis	
Samples	Concentration of samples	Zone of inhibition (mm)		
Standard	Gentamycin (80 mcg)	20	23	27
Venom Extrat	Negative control	-	-	-
	T1 (400 mcg)	-	-	-
	T2 (800 mcg)	10	-	-

Table:2- Anti-Bacterial Assay against Gram Negative Bacteria

Name of organisms used	Klebsiella pneumoniae	Pseudomonas aureus	Escherichia coli
Samples	Concentration of samples	Zone of Inhibition (mm)	

Standard	Gentamycin (80 mcg)	30	25	22
Venom Extract	Negative	-	-	-
	T1 (400 mcg)	-	-	-
	T2 (800 mcg)	15	14	-

DISCUSSION

Production of antimicrobial substances is not only an important aspect of innate individual immunity of organisms (Otti et al., 2014) but in social insects it represents also a significant component of the so called "social immunity" (Cremer et al., 2007; Schluns and Crozier, 2009). This is especially true when chemicals are used for preventing pathogens or opportunistic microorganisms to spread in the nest environment (Baracchi et al., 2011; Tragust et al., 2013; Tranter et al., 2013) and to attack defenceless brood.

In this study nasty red fire ant venom was taken as an alternative of antibiotic against certain bacteria. In this study, the ant venom extract showed antibacterial activity against the human pathogens. The antibacterial activity of venom extracts and antibiotics against six bacterial strains were done. The ant venom extracts and antibiotics produced a marked difference in antibacterial activity. The venom extract have shown inhibitory effect on the growth of the bacteria studied. It is due to ant venom extract contain certain active antibacterial compounds. This was similar to the finding of Sullivan et al., 2009, studied the antibacterial activity of synthetic fire ant venom. Specific isomers of synthetic fire ant venom alkaloids have antibacterial activity against human pathogens.

Based on Clint Penick et al., (2018) predicted that extracts from all ant species would inhibit growth of Staphylococcus epidermidis. Extracts from over half of the ant species they tested were inhibited bacterial growth, but 40% did not. The species exhibiting the strongest antimicrobial activity were broadly distributed across the ant phylogeny with one cluster in the tribe Solenopsidini (Monomorium minimum, Solenopsis invicta and S. molesta).

Just as ants evolved the ability to culture bacteria to produce antibiotics long before humans (Santoset al., 2004), it follows that indiscriminate use of antimicrobial secretions by ants could lead to the evolution of resistant diseases, and a drastic reduction in the efficacy of their biochemical arsenal, with a consequent decrease in relative fitness. Tara Devi et al., (2019) studied various ant species ant their medicinal uses. Based on the above study, the specific isomers of some synthetic fire ant venom such as solenopsin have shown antibacterial activities against the six types of bacteria.

According to this, the extracts of nasty red fire ant can be formulate a new natural antibacterial product for controlling infections. This study may thus lead to the formulation of new natural antibacterial agent and this may be beneficial in future prospects for mankind.

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