

Antifungal Efficiency of Haemolymph and Aqueous Extraction of Red Velvet Mite, *T. Grandissimum*

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Abstract

Antifungal activity of the extracts of whole red velvet mite, skin and fresh haemolymph was evaluated. It was very interesting to observe that all the above three components registered a good antifungal activity. Of the three products of mites tested, the fresh haemolymph registered the maximum antifungal activity. Next to the haemolymph, the alcohol extracts of the skin exhibited a high antifungal activity. A dose dependent variation was observed in the antifungal activity. The extracts of haemolymph at a dose level of 200 mg/disc showed the highest inhibitory activity.

Keywords: *T.grandissimum*, Haemolymph, Antifungal activity and pathogens

1. Introduction

Red velvet mites are arachnid arthropods inhabiting the subterranean habitats for over several million years and they protect their soft velvet like body and young ones in the soil by some special defense mechanism from soil borne microbes. According to Sharma (2003) since insects had evolved over 500 million years ago and flourish in all sorts of habitats, they must be manufacturing a wide assortment of compounds to counter microbes that threaten them. The *Trombidium* species are exposed to a cocktail of nasty bacteria and fungi so that their defense mechanism unleashes all its power against the pathogens. Antifungal activity of the other subterranean arthropods like termites had been well documented (Solavan 2004, Solavan *et al.*, 2007, Wilsanand *et al.*, 2007). Antimicrobial peptides had been reported from termites (Lamberty *et al.*, 2001) and ants (Andary *et al.*, 1996, Old Field 1989, Goodman 1986, Mackintosh *et al.*, (1998 and Diamond, 2001 Orivel *et al.*, 2001) Insects had been proved to be very important sources of drugs for modern medicine since they have immunological, analgesic, anti-bacterial, diuretic, anti-rheumatic and anesthetic properties (Yamakawa 1998). Beattie *et al.*, (1986) had stated that the arthropods that live on close proximity to each other such as wasps, bees, mole crickets, scarab larvae, cicadanymphs, and centipedes are subject to microbial attacks and epidemic diseases. To limit disease activity they incorporate antimicrobial compounds into their nests. Holldobler and Wilson (1990) reported that the soil dwelling ants use chemical defense against fungi and bacteria in their environment.

Oudhia had written (Forest information update (FIU) a free weekly email newsletter, No.21, 3 July 2000) that the current rate of red velvet mite, *Trombidium grandissimum* Koch in Chhattisgarh State of India was equivalent to

20 US Dollars / Kilograms, and these mites were used as good sex tonic and its oil is very useful in treating more than 50 common diseases. But there is no scientific study on the antimicrobial activity of the extracts of the red velvet mites. Hence in the present study attempt has been carried out to find out the antifungal and antifungal activities of the extracts of the whole mites, skin and fresh haemolymph.

2. Material and Methods

The human fungal pathogens such as *Aspergillus niger*, *A. fumigatus*, *Candida albicans*, *Phytophthora infestans* and *Trichophyton rubrum* were selected for antifungal screening. Ten microlitre of the fungal broth culture was aseptically transferred to the air dried sterile Sabouraud dextrose agar agar plates and spread the culture uniformly with the help of a sterilized spreader made up of glass rod.

The extracts of outer skin, whole body and fresh haemolymph were subjected to pilot study. A pilot screening of the extracts were carried out by impregnating a 6 mm sterile Whatmann number.1 filter paper discs. The disc was loaded with the extracts to give a final load of 100 mg/ disc. The discs were allowed to dry completely and after the aqueous solvent was evaporated, the discs were placed on the Petri plate previously seeded with the respective fungal strains. Three replicates were used for each treatment. Control discs were kept without any extracts but soaked in respective microlitre of aqueous solvent and dried plates were then kept at 37⁰ C in an incubator for 24hrs. The inhibition – zone width (distance from the edge of the paper disc to the outer edge of the inhibition zone) was measured to the nearest mm, at 24hrs by using Hi-Media antibiotic zone scale and expressed in standard deviation of mean ($\pm$ SE). The antifungal activity of fresh haemolymph, the extracts of skin and whole body were calculated for different concentrations.

3. Results and Discussion

Antifungal activity of the extracts of whole red velvet mite, skin and fresh haemolymph was evaluated. It was very interesting to observe that all the above three components registered a good antifungal activity. For the present investigation, five fungal organisms were selected and the results are presented in the Tables 1 - 3.

Of the three products of mites tested, the fresh haemolymph registered the maximum antifungal activity. Next to the haemolymph, the alcohol extracts of the skin exhibited a high antifungal activity. A dose dependent variation was observed in the antifungal activity. The extracts of haemolymph at a dose level of 200 mg/disc showed the highest inhibitory activity. Of the five fungal species tested the dermatophyte, *Trichophyton rubrum* responded much to the extracts of the skin and the haemolymph of the red velvet mite. Inhibition zone for *T. rubrum* was 8.5 ± 0.1 mm for haemolymph and 7.3 ± 0.2 for the skin extracts. This was followed by *Phytophthora infestans*. It showed an inhibition zone of 8.0 ± 0.2 for haemolymph and 7.2 ± 0.2 for skin extract. Antifungal Property was poor in *Aspergillus niger*. The inhibition zone for *Aspergillus niger* was 7.6 ± 0.2 mm for the haemolymph of red velvet mite and 5.5 ± 0.3 mm for the skin extract of red velvet mite.

The whole body extract of red velvet mite had lesser antifungal activity than the haemolymph and skin extract. Here also a dose level of 200 mg/ disc showed the highest inhibitory activity than 100 mg/disc and 150 mg/ disc. The whole body extracts showed a less inhibitory activity (3.8 ± 0.2 mm) against *T. rubrum* at a dose level 200 mg / disc. The response of *Candida albicans* was more to the whole body extract of red velvet mite. Inhibition zone for *C. albicans* was 6.6 ± 0.2 mm at a dose level 200mg/ disc. The antifungal activity in the extracts of the skin of velvet mite promote the possibility to develop new drugs for dermatitis. The rural people in Tirunelveli District, collect the mites and prepared its oil and apply this oil in disease affected (Eczema) areas in the skin.

It is widely accepted that plants, animals and their by-products used as a source of folk or traditional medicines indicate the presence of a biologically active constituent(s) in them. A significant portion of the currently available non-synthetic and/or semi-synthetic pharmaceuticals in clinical use is comprised of drugs derived from plants, animal, microbial, and mineral products (Soejarto, 1996). However many animals have been methodically tested by pharmaceutical companies as sources of drugs to the modern medical science (Kunin and Lawton, 1996). Approximately 109 animals and their 270 uses are reported in folk medicine in different part of India. The number of animals reported for medicinal purposes in different parts of India is enough to feel a need to discuss on the use of animals and their products, as medicines. In order to stress how important animals were, are and can be as sources of pharmacological substances and discussion on the use of the animals and their products, as medicines in conservation biology and sustainable use. We have concluded red velvet mites also one of the important zoo therapeutic agent for modern medicines. The antifungal potential in the haemolymph and whole body extracts of Red velvet mite suggests that the mite possess antifungal compounds and this has to be explored in future.

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Table 1. Antifungal activity of haemolymph of *T.grandissimum* against fungal pathogens in Disc plate method

Organisms	Zone of inhibition (mm) (± S.D)			Standard antifungal agent Myconazole
	Concentration of haemolymph(mg/disc)			
	100	150	200	50µg/ml
<i>Aspergillus niger</i>	4.3±0.3	5.2 ± 0.3	7.6 ± 0.2	20.3
<i>Aspergillus fumigatus</i>	4.4 ± 0.3	5.5 ± 0.3	8.1 ± 0.2	18.4
<i>Candida albicans</i>	4.5 ± 0.5	5.6 ± 0.1	7.5 ± 0.3	21.6
<i>Phytophthora infestants</i>	3.6 ± 0.4	5.2 ± 0.2	8.0 ± 0.2	20.5
<i>Trichophyton rubrum</i>	5.8 ± 0.2	6.7 ± 0.1	8.5 ± 0.1	20.2

Table 2. Antifungal activity of skin extracts of *T.grandissimum* against fungal organisms in Disc plate method

Organisms	Zone of inhibition (mm) (±S.D)			Standard antifungal agent Myconazole
	Concentration of skin extract(mg/disc)			
	100	150	200	50µg/ml
<i>Aspergillus niger</i>	3.6 ± 0.2	4.4 ± 0.2	5.5 ± 0.3	20.3
<i>Aspergillus fumigatus</i>	4.1 ± 0.3	4.6 ± 0.3	6.4 ± 0.3	18.4
<i>Candida albicans</i>	4.3 ± 0.2	5.5 ± 0.2	6.7 ± 0.3	21.6
<i>Phytophthora infestants</i>	4.7 ± 0.2	5.2 ± 0.3	7.2 ± 0.2	20.5
<i>Trichophyton Sp</i>	4.6 ± 0.1	5.4 ± 0.2	7.3 ± 0.1	23.4

Table 3. Antifungal activity of whole *T.grandissimum* extracts against fungal organisms in Disc plate method

Organisms	Zone of inhibition (mm) (± S.D)			Standard antifungal agent Myconazole
	Concentration of whole sample (mg/disc)			
	100	150	200	50µg/ml
<i>Aspergillus niger</i>	3.6 ± 0.2	4.5 ± 0.1	5.5 ± 0.1	20.3
<i>Aspergillus fumigatus</i>	3.7 ± 0.1	4.3 ± 0.2	5.7 ± 0.3	18.4
<i>Candida albicans</i>	3.8 ± 0.3	4.6 ± 0.3	6.6 ± 0.2	21.6
<i>Phytophthora infestants</i>	3.6 ± 0.2	3.7 ± 0.2	4.6 ± 0.2	20.5
<i>Trichophyton rubrum</i>	3.4 ± 0.1	3.5 ± 0.1	3.8 ± 0.2	23.4