

RESEARCH ARTICLE

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Corresponding Author

J. Sherly Carolyn

Department of Zoology, Scott
Christian College, Nagercoil,
TamilNadu, India.

Email: sherlycarolyn@yahoo.com



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Antimicrobial activity of the egg white extract of the blister beetle, *Mylabris pustulata* (Thunberg).

J. Sherly Carolyn and D. Selva Raj

Department of Zoology, Scott Christian College, Nagercoil, TamilNadu, India.

ABSTRACT

The antibacterial activity of the egg white extract of the blister beetle *M. pustulata* was evaluated against four gram-positive and five gram-negative bacteria. Maximum activity was observed against *V. parahaemolyticus* and *B. subtilis* with the zones of inhibition of 25.33 ± 0.47 mm which was higher than that of a commercial antibiotic disc. Minimum activity was observed against *P. aeruginosa* and *P. vulgaris* with both having the zone of inhibition of 15.33 ± 0.47 mm

Introduction

Unlike vertebrate innate immunity, insects innate immunity is non-specific in nature and they lack any immunological memory [1]. In general insects immunity is divided into two broad groups. The first one is non-specific immunity which consists of structural and passive barriers and specific immune system involving cellular and humoral immunity. Cellular immunity includes chemical reactions and humoral immunity involve activation of prophenol-oxidase cascade and induction of immune proteins such as lysozymes, lectins and anti-bacterial and anti-fungal proteins [1, 2, 3].

For about six decades the antibiotics seemed to work wonders with bacterial infections and presumed to be a promising solution for serious infectious diseases. But bacterial resistance to antibiotics is becoming a very serious problem since more and more bacteria have developed resistance to more and more antibiotics over these years [4].

Natural products have been demonstrated to be an alternative source for antibiotics as there have been significant progress in the discovery of new compounds from natural sources with antimicrobial activity [5]. Since 1981, when the first antimicrobial peptide was isolated from the giant silkworm *H. cecropia*, insects have become the important source an antimicrobial peptides [6].

The blister beetle *M. pustulata* (Thunberg) belongs to the family Meloidae. It is one of the larger species of the family. This phytophagous insect is distributed throughout India and it feeds extensively on the flowers of many crop plants.

M. pustulata is about 25mm in length and the adults have very obvious black and red colouration [7]. *M. pustulata* causes severe blisters on the skin due to the presence of a chemical cantharidin in their body fluids [8].

This study aims at the evaluation of antibacterial activity of the whole body extract of the blister beetle, *M. pustulata* against selected gram-positive and gram-negative bacterial pathogens using egg white as extraction solvent. To the best of our knowledge this is the first of its kind in this blister beetle, *M. pustulata*.

Materials and methods

Specimen collection and Identification:

M. pustulata beetles were collected from two stations (Mylady and Scott Christian College premises). The beetles were handpicked from the plants during dusk and brought to the laboratory and defrosted at -20°C for further analysis.

Preparation of insects extracts:

The beetles were air dried in a hot air oven at 50°C . The extraction procedure was followed by Ma and Ruan, 2008. The air dried insects body of *M. pustulata* was soaked in the hen's egg white in a conical flask for 24h and then was removed from the egg white. The egg white was then mixed with the chloroform (20ml) and filtered through Whatman No.1 filter paper to make the final extract and was used for assaying antibacterial activity.

Antibacterial assay:

Test Microorganisms:

Four gram-positive bacteria including *S. aureus*, *B. subtilis*, *E. aerogenes* and *M. luteus* and five gram

negative bacteria including *P. aeruginosa*, *V. parahaemolyticus*, *E. coli*, *K. pneumoniae* and *P. vulgaris* were used for antibacterial screening assays. Stock cultures of the microorganisms were grown in nutrient agar slants at 37°C and were subcultured and maintained in nutrient broth at 40°C.

Evaluation of antibacterial activity:

Agar Disc Diffusion Assay:

Antimicrobial activity of each solvent extract was determined using a modified disc diffusion method (9). Pathogenic bacterial strains were inoculated in sterile nutrient broth and incubated at 37°C for 24 hours. Sterile Mueller Hinton agar plates were prepared and allowed to set. The pathogens to be screened were

swabbed on top of the solidified media using sterile swabs. Then 25µl of the extract was pipetted on a 6mm sterile paper discs. The solvent was allowed to evaporate and the disc was placed on the surface of the plate. The plates were incubated at 37°C for 48 hours. After incubation, the inhibition zone was measured. Assay was carried out in triplicate and control plates were maintained. The antibiotic disc Sparfloxacin was used as the positive control and solvent discs were used as the negative control. Zone of inhibition was measured from the edge of the disc to the clear zone in millimeters.

Results

Sl.No	Gram-positive bacteria	Egg white extract	Sparfloxacin Positive control	Chloroform Negative control	Egg white Negative control
1	<i>S. aureus</i>	18.33 ± 0.47	16.67 ± 0.47	0.67 ± 0.47	5.33 ± 0.47
2	<i>B. subtilis</i>	25.33 ± 0.47	20.67 ± 0.47	0.67 ± 0.47	2.33 ± 0.47
3	<i>E. aerogenes</i>	18.67 ± 0.47	20.67 ± 0.47	0 ± 0	5.33 ± 0.47
4.	<i>M. luteus</i>	19.67 ± 0.47	17.67 ± 0.47	0 ± 0	6.67 ± 0.47

All values are Mean ± Standard deviation

Table: 1 Effect of egg white extract of *M.pustulata* against Gram-positive bacteria (Zone of inhibition in mm).

Sl.No	Gram-negative bacteria	Egg white extract	Sparfloxacin Positive control	Chloroform Negative control	Egg white Negative control
1.	<i>P. vulgaris</i>	15.33 ± 0.47	18.67 ± 0.47	0 ± 0	2.33 ± 0.47
2.	<i>K. pneumoniae</i>	18.33 ± 0.47	20.67 ± 0.47	0.67 ± 0.47	1.67 ± 0.47
3.	<i>P. aeruginosa</i>	15.33 ± 0.47	18.33 ± 0.47	0 ± 0	0.67 ± 0.47
4.	<i>V. parahaemolyticus</i>	25.33 ± 0.47	18.67 ± 0.47	0 ± 0	0 ± 0
5.	<i>E. coli</i>	22.67 ± 0.47	18.67 ± 0.47	0 ± 0	2.33 ± 0.47

All values are Mean ± Standard deviation

Table: 2 Effect of egg white extract of *M.pustulata* against Gram-negative bacteria (Zone of inhibition in mm).

The results revealed that egg white extract of the *M. pustulata* showed a broad spectrum activity against the tested pathogens. It inhibited the growth of both gram-positive and gram-negative bacteria.

Maximum activity was displayed against *V. parahaemolyticus* and *B. subtilis* with the clear zone of inhibition of 25.33 ± 0.47 mm. The extracts displayed a strong antibacterial activity towards *E. coli* (22.67 ± 0.47mm) and *M. luteus* (19.67 ± 0.47) mm. These zones of inhibition were higher than that of the corresponding values of the antibiotic Sparfloxacin.

The extracts showed a good antibacterial activity towards *E. aerogenes* (18.67 ± 0.47mm) followed by *K. pneumoniae* and *S. aureus* with the zones of inhibition of 18.33 ± 0.47 mm. These values were in fact lower than that of the antibiotic but for *S. aureus*. Minimum activity of the egg white extracts was observed against *P. aeruginosa* and *P. vulgaris* with the zone of inhibition of 15.33 ± 0.47mm.

The egg white and the chloroform solvent which were used as the negative control showed low and restrained activity compared to that of the egg white extract and the antibiotic.

Discussion

The results of the antibacterial activity of the egg white extract of the blister beetle, *M. pustulata* revealed that the extracts is versatile, being capable of inhibiting all the types of pathogens tested and the zones of inhibition lasted for a week.

The egg white extract of the *M. pustulata* showed that the antibacterial activity of the egg white extracts was higher than that of the antibiotic Sparfloxacin against some pathogenic bacteria. The whole body extracts of some insects such as the red velvet mite *T. Grandissimum* [10] and the larvae of the insect *Z. morio* [11] showed antibacterial activity against all the pathogens tested and some insects displayed selective antimicrobial activity against tested pathogenic organisms such as the methanolic extracts of the adults of the red palm weevil *R. ferruginus* [12].

But using egg white as a solvent for testing biological properties for samples will turn out to be a boost for pharmacologists. Egg white can be used as a two phase solvent because it easily dissolves the water-soluble as well as lipid-soluble ingredients and it is easily absorbed by the patients [13].

The predominant antibacterial activity displayed by egg white extract of *M. pustulata* may be due to the effectiveness of egg white in extraction of both water soluble and lipid soluble antimicrobial component present in the body of *M. pustulata*.

There is no such work on the blister beetle, *M. pustulata* as well as on other blister beetles of India. So there is little literature on this study. *Mylabris* species are still employed in traditional Chinese medicine and interest in the clinical use of cantharidin as an antitumor agent has been growing owing to the reports that cantharidin produce cytotoxic effects in a number of human tumor cell lines and primary tumor cells [14, 15]. Cantharidin from the *Mylabris* species had earlier been found to function in killing pests and weeds as an antiviral and antibiotic and useful in plant protection [16, 17, 18].

So further study on the testing of this egg white extract against a more wide range of microorganisms and purification and isolation of the active principle behind it is warranted.

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