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Ladybird Larvae, Coelophora bisselata Mulsant (Coleoptera: Coccinelladae) as a source of fibrinolytic enzyme

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Abstract

The combination of traditional knowledge and advanced biotechnology can explore the bio-prospects of biological resources as a new source of drug or medicine and thus can promote the sustainable utilization of biodiversity. Insects belong to a diverse Class and thus their biodiversity can be contributed for exploitation of incredible variety of bio active ingredients. Though there have been few studies on the antibacterial potential of ladybird species, no report is being made on the fibrinolytic activity of ladybird larvae till date. In the present study, we for the first time evaluated the *in vitro* fibrinolytic activities of crude extracts from larvae as well as adult ladybird, *Coelophora bisselata*. The ladybird, *C. bisselata* (Coleoptera: Coccinelladae) is a cosmopolitan polyphagous predatory beetle which is often used as biocontrol agents against many economically important pest species. The fibrinolytic activity was tested using fibrin plate method. Results showed that larvae extract possessed profound fibrinolytic activity. However, in the extract from adult form no such activity was observed. The study suggested that crude extracts from larvae of *C. bisselata* might prove to be useful alternative source for the development of new drugs for treatments involving blood coagulation and fibrinolysis.

Key words : Insect crude extract, fibrinolytic activity, *Coelophora bisselta*

INTRODUCTION

Ladybird beetle, *Coelophora bisselata* Mulsant (Coleoptera: Coccinelladae), predominantly found in the Indian agro ecosystem, is a cosmopolitan predator that feed on diverse range of insect species that are considered serious pests of many economic important plants[1-2]. Hence, *C. bisselata*, is often considered as a good biocontrol agent and has immense importance in integrated pest management strategies[3-4].

There have been previous studies on the antimicrobial potential of some species of ladybird beetles. Though Harmonin developed from ladybird, *Harmonia axyrdis* is known to be active against tuberculosis and malaria pathogen, some other species of ladybird viz. *Coccinella septempuncta* and *Adalia bipunctata* showed insignificant effect[5]. Also, there is a report on the ethno-medicinal use of ladybird as cure for anaemia by the tribes of South India[6]. However, ladybird *Coelophora bisselata* has never been studied before as a source of therapeutics for myocardial and cerebral thrombosis.

Thrombotic disorders including cerebral stroke and myocardial infarctions have become a serious concern all over the world. Commonly used fibrinolytic agents for thrombotic disorders include streptococcal streptokinase, urokinase and human tissue type plasmin activator (t-PA)[7]. High doses of these drugs often lead to bleeding complications like rapid thrombolysis which might result in systemic generation of plasmin leading to rapid decline of clotting factors and other adverse effects[8]. Discovery of effective thrombolytic agents from alternative sources devoid of the adverse effects can therefore significantly open up new dimension in the treatment of thrombotic disorders. Therapies with thrombolytic agents have been identified and characterized from micro organisms[9] and annelids like leech[10] and earthworm[11-12] as well as from snake venoms[13-14]. Insect sources like venome of bumblebee, *Bombus sp.*[15] , dung beetle, *Catharsius molossus*[16] and lepidptera, *Lonomia oblique*[17] were also studied and found to be effective. However, the fibrinolytic properties of ladybird beetle have never been explored and investigated before. Therefore, the aim of the present study was to obtain alternative source for fibrinolytic agent for therapeutics from *C. bisselata*, a naturally occurring insect.

MATERIALS AND METHOD

Collection of Specimen

Both larvae and adult *C. bisselata* were collected from chili plants found in the agricultural farms at different locations, in and around Agartala, northeast India (23.50° N latitude, 91.25°E longitude).

Preparation of insect extract and protein estimation

The whole body of the insect larvae as well as the adult beetle were weighed and washed with autoclaved deionized water and crushed separately in Phosphate Buffer Solution (pH 7). Both the insect extracts were stored in -20ºC for future use. Protein concentration was determined using Lowry method[18].

Preparation of fibrin plate and the experimental design

Fibrinolytic activity of the extract was carried out by fibrin plate method[19]. Both the crude larvae extract and the adult extract were applied directly into a small pore created in the petriplate containing artificial fibrin. 15 µl of larvae extract was used in well nos. 1 and 30 µl of larvae extract was used in well no. 2 while 15 µl and 30 µl of adult extract was applied in well nos. 3 and 4 respectively. Streptokinase was used as a positive control (well nos. 5 and 6) while phosphate buffered saline (PBS) served as negative control (well no. 7). The petriplate was incubated at 37ºC and observed at various lengths of time. The fibrinolytic activity was quantified by measuring the lysis area on the plate.

RESULT

Result obtained in the form of clear zone in fibrin plate is presented in Fig no. 1. Equivalent amount of protein was loaded in

the respective wells for larvae and adult extracts. No clear zone was observed around the wells containing the adult extracts (well no. 3 and 4). However, clear and distinct zone appeared around the wells containing the larvae extract (well nos. 1 and 2). The diameter of the circles proportionately increased with the increase of incubation time. Clear zone appeared around positive control (well no. 5 and 6) where as in negative control well (well no. 7), containing PBS, no zone appeared as expected.

Figure no. 2 depicts the comparison of the response of crude larvae extract with that of the response of control in time dependent manner. At the given protein concentration (31 ug/µl), the response of the crude extract was found to be little lower in comparison to that of Streptokinase. However, the pattern of response was found to be similar. Regarding the pattern, in both the cases, fibrinolytic activity gradually increased in the first 12 hours. Thereafter, there was a sudden surge in the activity. The fibrinolytic activity of both the control and experimental extract was found to be directly proportional to the time elapsed. No record could be maintained with the extract of the adult beetle as it showed no fibrinolytic activity.

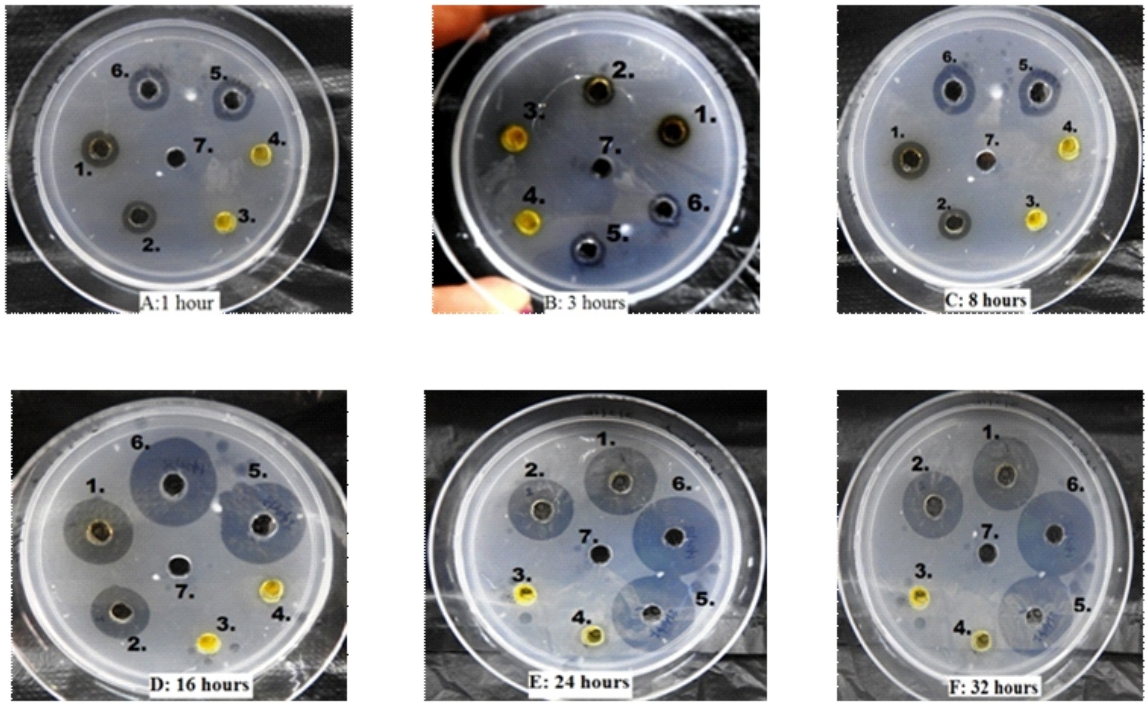

Fig 1: Comparative fibrinolytic properties of the extracts of larvae of *C. bisselata,* (well no. 1 and 2) and that of adult *C. bisselata* (well no. 3 and 4). Well no. 5 and 6 represent positive control and well no. 7 represent negative control.

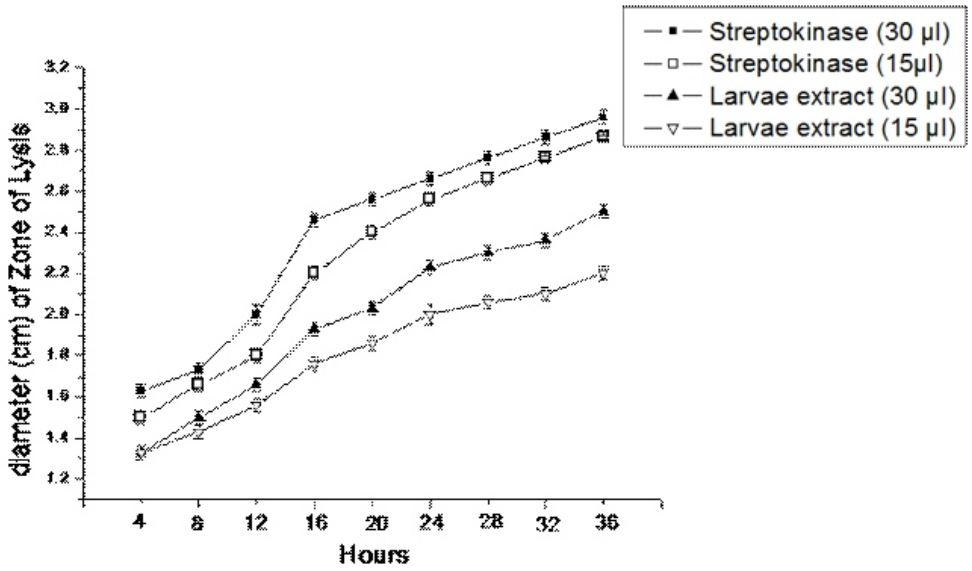

Fig 2: Incubation effect (fibrinolysis) of C. bisselata extract on fibrin plate

DISCUSSION

Thrombolytic disorders including cerebral stroke and myocardial infarctions are rising on an alarming rate throughout the world. Though some drugs are already available in market, search for more effective thrombolytic therapeutics is essentially going on to combat the situation. There have been constant researches on alternative sources of fibrinolytic agents from plant as well as animal origin[20]. There were previous studies on identification and characterization of thrombolytic agents such as nattokinase in vegetable cheese natto from micro organisms[21], lumbrokinase from earthworms[22], lonofibrase from lepidopteran larvae of *Lonomia* sp.[23] etc. Some other insects that were widely studied in this regard include mantis[24], honey bee and bumble bee[25] and dung beetle[26]. Most of these studies demonstrated the presence of serine protease as key ingredient in the extract that was responsible for the fibrinolytic activity. The fibrinolytic activity shown in the present study by the extract of larvae of ladybird, *C. bisselata* may also be due to the presence of serine protease. As observed, the extract of adult stage of *C. biselatta* showed no activity. But the extract of larval stage showed profound fibrinolytic activity. This could be due to the stage specific expression of the enzyme. The expression of the enzyme may get ceased or reduced at adult stage. Previous studies on specific proteinase activity of Colorado potato beetle, *Leptinotarsa decemlineata* was found to be much higher in larvae midgut extracts in comparison to adults[27]. Gut proteinase activity in *Helicoverpa armigera* was found to increase gradually from first to fifth instar stages after which there was a sharp decline in the sixth instar[28].Variation in diet in different stages of life might also contribute to the diversity in the proteinase activities and flexibility in their expressions in insects.

So far no report is available on the fibrinolytic activity of *C. bisselata* and this study could be the first report in this regard. The study warrants further purification and characterization of the enzyme.

CONCLUSION

This is a first report suggesting that crude extracts from larvae of ladybird, *C. bisselata,* might prove to be useful alternative source for the development of new drugs for treatments involving blood coagulation and fibrinolysis. Further studies can now be designed to characterize and identify the protein of the insect extract involved in fibrinolytic activity.

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