Evaluation of Antisickling Activity of Some Insect Extracts from Katanga in Democratic Republic of the Congo

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ABSTRACT

Democratic Republic of the Congo boasts a large animal and vegetal biodiversity that can be used to alleviate the pain of people affected by sickle cell disease. If some local plant species were tested for their antisickling activity, no Congolese insect species were subjected to this kind of study. In this work, some insect species from Katanga province were collected and their methanol extracts tested for antisickling activity. Nine insect species among which two are edible were identified. They belong to eight families and three orders. The Lepidoptera order contains 55.6% of studied species. The evaluation of the in vitro antisickling activity indicates that Chrysirida madagascariensis specie, inedible caterpillars of the family of Psychidea and order of Lepidoptera, showed the highest activity with an inhibition rate of about 60%. This indicates that there could be other insect species with interesting antisickling activity like some plant species whose biological activity has been proven.

Keyword: Insects, antisickling activity, Lepidoptera, Psychidea, Chrysirida madagascariensis

INTRODUCTION:

Sickle cell anemia affects over 50 million people. Each year two hundred children are born anemic. According to WHO estimates, around 300 to 500,000 children are born each year with a hemoglobinopathy of which 80% in developing countries, particularly in Africa [1-4]. In Democratic Republic of the Congo (DRC), it is estimated that there are about 25 to 30% of healthy carriers (AS) and about 2% of homozygous (SS) [5, 6].

In fact, sickle cell anemia is an inherited blood abnormality hitherto incurable, due to the presence of hemoglobin S. Many practices have been developed to fight against this disease. These include the management of vaso-occlusive crises, bone marrow transplantation, repeated transfusions. Unfortunately, these treatments were shown to be expensive or unreliable [1, 2, 6].

Some studies have been carried out on the plants and the results are highly encouraging [3, 6, 7-13]. These studies have shown that plants would possess certain chemical substances capable of normalizing deformed erythrocytes [14-16]. It can be noticed that plants are not the only source of natural bioactive substances, there are also insects and some animals. As a matter of fact, insects contain interesting immunomodulating substances and secondary metabolites which are currently designed for the search for new molecules to therapeutic activities [17-22].

In spite of the ravages of some insect species such as culicids, disease vectors, other insects occupy a prominent place in all environmental media and ecosystems. They are necessary for the survival of many species, animals as well as plants. For example, bees lay upon flowers the essential pollen reproduction, clean waters and contribute to the fermentation and putrefaction. Ladybug protects plants by removing the aphids [23].

Insects also play a vital role in the decomposition of corpses, excrements and dead plant material. This is the case of the herdsmen, of which the larvae feed on animal feces. The Secretion substances of certain species such as Diamphidia simplex were used as an arrow poison [23, 24]. Without insects, many food chains would be broken. They are also a source of protein highly coveted by some people who eat shrimp, flying ants, termites, beetles, grasshoppers, locusts and crickets [24]. Moreover, the insects were also the subject of medicinal uses in some traditions [17-23]. This assay was carried out in order to evaluate the in vitro antisickling activity of some insect extracts by assessing their effect on the blood sickling of SS erythrocytes.

MATERIALS AND METHODS

Biological Material

The biological material consists of nine species of insects, namely Anaphe panda (Notodontidae: Lepidoptera), Elaphrode latae (Notodontidae: Lepidoptera), Chrysirida madagascariensis, Diaphone eumela, Aspidoproctus maximus, Oecophila smaragdina (Formicidae: Hymenoptera), Epilachma tibialis (Epilachninae: Coleoptera), Cheilomenes sulphurea (coccinellines: Coleoptera) and Exochomus concavus (Chilochorinae: Coleoptera).

The insects were collected during the period from September 2010 to June 2011 in Katanga, a province of the DRC. They were identified by Mr Kisimba of the Faculty of Sciences at the
University of Lubumbashi. Caterpillars have been collected on the host plant species in the gallery forest of Miombo in the vicinity of the city of Lubumbashi while ants came from Kamina. The specie Epilachna tibialis was collected in Fungurume at 200km far from Lubumbashi on the road to Kolwezi while Cheilomenes sulphurea and Exochomus concavus were collected in the surrounding of Lubumbashi and Kipushi.

Extraction
Collected insects, were washed four times to the exhaustion and then dried at room temperature (approximately 27°C), ground afterwards soaked in methanol for 24 hours. The solution was filtered through Whatman paper and the filtrate was concentrated on a rotary evaporator at room temperature and at -0.8 bar. The resulting residue was dried in the open air and weighed in order to calculate the yields. The resulting crude extract was stored in the refrigerator in order to be used for biological tests.

Preparation of extract solutions
Each dry extract of insects (0.2g) have been dissolved by adding a drop of DMSO and 50ml of saline solution.

Blood conditioning
Blood taken from a sickle cell anemia person is kept in a test tube containing a standard anticoagulant, EDTA and kept in the fridge at 4°C.

Emmel’s test
The sickling test was performed in the same manner as for the plant extracts as previously described [25-28]. The solutions of insect extracts have been prepared using saline solution (0.9%) of pH 7.4. The number of erythrocytes standardized relatively to SS control was determined using a hand counter.

Evaluation of normalization rate of sickle cells
The biological activity of insect extracts can be expressed as an antiscickling activity or inhibition of sickling rates by using the following formula [6, 25-32]:

\[
\text{Normalization rate ou TIF} (%) = \frac{(\text{NDAV} - \text{NDAP})}{\text{NDAV}} \times 100
\]

With NDAP: Number of sickle cells after treatment
NDAV: Number of sickle cells before treatment
TIF: inhibition of sickling rate.

RESULTS AND DISCUSSION
Insect identification, location and use

The collected insects and their identification are shown in table 1

<table>
<thead>
<tr>
<th>No</th>
<th>Species (Order and Family)</th>
<th>Vernacular Name</th>
<th>Location</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Anaphe panda (Lepidoptera and Notodontidae)</td>
<td>Masalya (tsokwe), Finamisia (Bemba)</td>
<td>Pseudolachnostylis maprouneifolia (Kasungami, Kashamata)</td>
<td>Edible Caterpillar</td>
</tr>
<tr>
<td>02</td>
<td>Libyaspis wohberbergi (Lepidoptera and Margodidae)</td>
<td>Bilulu (Swahili)</td>
<td>Acacia auricouliformis (Lubumbashi)</td>
<td>Non-Edible Insect</td>
</tr>
<tr>
<td>03</td>
<td>Chrysiridia madagascariensis (Lepidoptera and Psychidae)</td>
<td>Tebebela (Lala, Lamba) motolanda (Bemba)</td>
<td>Psidium goyava (Kikontwe Farm)</td>
<td>Non-Edible Caterpillar</td>
</tr>
<tr>
<td>04</td>
<td>Diaphone eumela (Lepidoptera and Noctuidae)</td>
<td>Mishi (Swahili)</td>
<td>Sphenostylis marginata (Kasungami)</td>
<td>Non-Edible Caterpillar</td>
</tr>
<tr>
<td>05</td>
<td>Elaphrode lactea (Lepidoptera and Notodontidae)</td>
<td>Tunkubi (Swahili) Utunkubi (Lala)</td>
<td>Brachystegie boehmi (Ferme Munongo)</td>
<td>Edible Caterpillar</td>
</tr>
<tr>
<td>06</td>
<td>Oecophila smaragdina (Hymenoptera and Formicidae)</td>
<td>Matetemena (Luba) Buyiri (Nande)</td>
<td>Fruit trees in the Northern of Katanga (Kamina)</td>
<td>To treat coughing, child convulsion, Edible Insect</td>
</tr>
<tr>
<td>07</td>
<td>Epilachna tibialis (Coleoptera et Epilachninae)</td>
<td>Kabandakwe (Swahili)</td>
<td>Solanum incanum (Fungurume Rock)</td>
<td>Non-edible Insect</td>
</tr>
<tr>
<td>08</td>
<td>Exochomus concavus (Coleoptera and Chilochorinae)</td>
<td>Kabandakwe (Swahili)</td>
<td>Thitonia diversifolia, Vernonia amygdalia in Lubumbashi</td>
<td>Non-Edible Insect</td>
</tr>
<tr>
<td>09</td>
<td>Cheilomenes sulphurea (Coleoptera and Coccinellinae)</td>
<td>Kabandakwe (Swahili)</td>
<td>Vernonia amygdalia in Lubumbashi</td>
<td>Non-Edible Insect</td>
</tr>
</tbody>
</table>

The distribution of insects according to orders is shown in Figure 1, below:
Figure 2 provides the edible and non-edible insects.

Figure 2. Edible and Non-Edible Insects

Extraction performance and characterization of crude extracts

The extraction yield of the insects and the characterization of the various extracts are given in Table 2.

Table 2. Performance and characterization of different crude extracts of studied insects

<table>
<thead>
<tr>
<th>Species</th>
<th>Weight of the sample (g)</th>
<th>Weight of the extract (g)</th>
<th>Performance (%)</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaphe panda</td>
<td>7.28</td>
<td>2.18</td>
<td>30.2</td>
<td>Brown</td>
</tr>
<tr>
<td>Aspidoproctus maximus</td>
<td>5.22</td>
<td>0.22</td>
<td>4.2</td>
<td>Red</td>
</tr>
<tr>
<td>Cheilomenes sulphurea</td>
<td>6.30</td>
<td>1.30</td>
<td>20.6</td>
<td>Red</td>
</tr>
<tr>
<td>Chrysirida madagascariensis</td>
<td>6.54</td>
<td>1.54</td>
<td>23.5</td>
<td>Red</td>
</tr>
<tr>
<td>Diaphone eumela</td>
<td>5.47</td>
<td>0.47</td>
<td>8.5</td>
<td>Brown</td>
</tr>
<tr>
<td>Elaphrode eumela</td>
<td>7.92</td>
<td>2.92</td>
<td>36.9</td>
<td>Yellow</td>
</tr>
<tr>
<td>Epilachna tibialis</td>
<td>5.10</td>
<td>0.10</td>
<td>1.9</td>
<td>Brown</td>
</tr>
<tr>
<td>Exochomus concavus</td>
<td>5.79</td>
<td>0.79</td>
<td>13.6</td>
<td>Red</td>
</tr>
<tr>
<td>Oecophila smaragdina</td>
<td>5.91</td>
<td>0.91</td>
<td>1.7</td>
<td>Red</td>
</tr>
</tbody>
</table>

Antisickling activity

The normalization rate of sickle cell persons or sickling inhibition by insect extracts is shown in Table 3 below.

Table 3. Rate of normalization of sickle cells or sickling inhibition

<table>
<thead>
<tr>
<th>Nº</th>
<th>Name of the insect species</th>
<th>NDAV</th>
<th>NDAP</th>
<th>TND/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anaphe panda</td>
<td>32</td>
<td>31</td>
<td>3.1</td>
</tr>
<tr>
<td>2</td>
<td>Aspidoproctus maximus</td>
<td>21</td>
<td>21</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>Cheilomenes sulphurea</td>
<td>75</td>
<td>75</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td>Chrysirida madagascariensis</td>
<td>75</td>
<td>30</td>
<td>60.0</td>
</tr>
<tr>
<td>5</td>
<td>Diaphone eumela</td>
<td>74</td>
<td>74</td>
<td>0.0</td>
</tr>
<tr>
<td>6</td>
<td>Elaphrode lactea</td>
<td>47</td>
<td>42</td>
<td>10.6</td>
</tr>
<tr>
<td>7</td>
<td>Epilachna tibialis</td>
<td>62</td>
<td>55</td>
<td>11.2</td>
</tr>
<tr>
<td>8</td>
<td>Exochomus concavus</td>
<td>56</td>
<td>54</td>
<td>3.5</td>
</tr>
<tr>
<td>9</td>
<td>Oecophila smaragdina</td>
<td>75</td>
<td>73</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>Negative control (saline solution 0.9%)</td>
<td>75</td>
<td>75</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Legend : NDAP : Number of sickle cell anemia after treatment
NDAV: Number of sickle cell anemia before treatment
TND : Normalization rate of sickle cell anemia

The distribution of insect extracts according to the antisickling activity is shown in figure 3.

Figure 3 : Distribution of insect extracts according to the antisickling activity

DISCUSSION

Table 1 results show that the insects belong to eight families, namely: Formicidae, Muscidae, Dolichoderidae, Notontidae, Psychidae, Margodidae, Chilochorinae and Cocconelliniae. These families belong to three orders: Lepidoptera, Coleoptera and Hymenoptera. Figure 1 shows that 55.6% of insects used were from Lepidoptera or five species out of nine. Hymenoptera are the least represented with 11.2% i.e. only one specie out of nine. Among insects collected some are edible while others are not. Figure 2 shows that of the nine insects which the activity of extracts was tested, only two insects are edible. It is Anaphe panda and Elaphrode lactea both Lepidoptera. It should also be noted that apart from Tachypodoiulus sp. no other insect species is reported to have antisickling properties in Congolese traditional medicine [33].

It is shown that in Table 2, all insect extracts are colored, this indicates that all insects have chromophore groups. The coloration of the extracts is red, brown or yellow. The highest yields were obtained for Elaphrode eumela extracts (36.9%) and Anaphe panda (30.2%) while the lowest yields were obtained for Oecophila smaragdina (1.7%), Epilachna tibialis (1.9%) and Aspidoproctus (4.2%). This means that the soluble substances in methanol are more abundant in Elaphrode eumela and Anaphe panda species than in other species. It can be noticed that the bioactive chemical groups for plant species were mostly found in polar solvents of which alcohols [3-6, 13-16, 25-32].

Evaluation of crude extract sickling inhibition rate of nine species used, shows the inhibition of sickling rate between 0 and 60%. The species Chrysirida madagascariensis has the strongest antisickling activity with 60% of normalization rate. For the remaining extracts, their inhibition rate is between 9 and 12% respectively for Epilachna tibialis (11.2%) and Elaphrode lactea (10.6%), and between 2 and 5% Exochomus concavus (3.5%) and Oecophila smaragdina (2.6%). Figure 3 shows that 33.3% of insect extracts showed no activity on the sickle cells; 55.5% of insect extracts showed a very low normalization rate of sickled erythrocytes and only 11.2% of extracts tested, or one out of nine, have shown an inhibition rate of sickling greater than 50%

It should be noted that some plant species of DRC that were tested, showed normalization rates of up to 90% [13-16,25-32]. But in most cases, these plant species have been reported as being used in traditional medicine against Sickle cell aneamia. On the contrary, there is no indication in the literature review concerning the insect antisickling activity in Congolese traditional medicine. As Chrysirida madagascariensis has shown an antisickling activity greater than 50%, it is encouraging and indicates that some insects could have a significant antisickling activity. Therefore, it is
necessary to continue to dig in this line trying to discover in the Congolese biodiversity both plant and animal, species that can be used by poor population to manage this chronic disease.

CONCLUSION

This study shows that although the insects are not quoted in the fight against sickle cell anemia in Congolese traditional medicine, *Chrysirida madagascariensis*, a species out of the nine of which the biological activity was assessed, showed the antisickling activity with 60% as a rate of sickling inhibition. This fact indicates that other insects can be found with an equal or greater effect. A bioguided splitting of *Chrysirida madagascariensis* extracts and even with other insects that showed less activity can lead to the isolation of active compounds against sickling.

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