EFFECT OF SOLVENTS ON EXTRACTION OF BIOACTIVE MASS PRESENT IN ABRUS PRECTORIUS LINNAEUS RESPONSIBLE FOR BODY WEIGHT REDUCTION IN ALBINO RATS

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ABSTRACT
Effect of solvents on extraction of bioactive mass present in Abrus precatorius Linnaeus leaves responsible for body weight reduction in albino rats was studied. Five solvent systems were used. They were, 10 : 1 (v/v) ethanol – chloroform mixture, 10 : 1 (v/v) methanol – chloroform mixture, 10 : 1 (v/v) acetone – chloroform mixture, 10 : 1 (v/v) petroleum ether – chloroform mixture and 10 : 1 (v/v) acetone – petroleum ether mixture. In all cases extraction was carried out with same amount of solvent system at room temperature for a period of 30 minutes. Results showed that mass obtained after extraction of leaves of A. precatorius L. with 10 : 1 (v/v) acetone – chloroform mixture had maximum body weight reduction activity in albino rats.

Key words: Extraction process, Body weight reduction activity, Abrus precatorius Linnaeus.

INTRODUCTION
In analysis of medicinal plants, extraction is a very crucial step and necessary to obtain the targeted active compound(s) responsible for the desired effect (Fabricant DS and Farnsworth NR, 2001). Extraction is also needed for further separation and characterization of the active compound. Various methods such as heating under reflux, sonification, soxhlet extraction etc. are used for extraction of plant samples (Pharmacopoeia, 2000; The Japanese Pharmacopeia, 2001; United States Pharmacopeia and National Formulary, 2002).

In extraction process solvents or mixture of solvents are used. Selection of solvent system largely depends on the specific nature of the bioactive compound being targeted. If the targeted compound is lipophilic in nature, dichloromethane or a mixture of dichloromethane / methanol are used. In case of hydrophilic compounds polar solvents such as methanol, ethanol or ethyl-acetate are used (Cosa P et al., 2006; Ghosh MN, 2005). However, final selection of the solvent system depends on maximum extraction capacity of the bioactive compound by the solvent systems. Selection of solvent system is, therefore, through trial and error.

Recently we found that plant Abrus precatorius L. could exert body weight loss in albino rats. Results are under communication. We intended to isolate the active compound responsible for the said activity. In connection with the isolation work we studied effects of different solvent systems to obtain active fraction. In this communication experimental details and results are being reported.

MATERIALS AND METHODS
Plant material
Leaves of A. precatorius L. were collected in morning hours (9 – 10 AM) from the medicinal plants garden of the University of North Bengal, Dist.
Darjeeling, west Bengal, India during the periods July – August as we have noted that leaves of *Abras precatorius* L. had maximum body weight loosing property during this period. Results are under communication. Leaves were authenticated by the experts of the department of Botany of the said University. A voucher specimen was kept in the department of Medical Biotechnology, Sikkim Manipal Institute of Medical Sciences of Sikkim Manipal University, Gangtok, Sikkim, India for future references. Leaves were sundried and powdered. The powder was used as the test drug.

**Animals**

Male Wister strain rats, body weight between 35 and 40g, were used for this study. Animals were housed individually in polypropylene cages, maintained under standard conditions like 12h light and 12h dark cycle, 20 - 30 degree centigrade, 35 - 60 % humidity. The animal experiment was approved by the ethics committee of the Institute. Rats were fed with standard rat pellet diet (Hindustan Lever Ltd.,Mumbai, India) and provided water ad libitum.

**Acute oral toxicity study**

Acute toxicity studies were carried out on Swiss albino mice by the method of Ghosh (Ghosh MN, 2005). Powdered leaves of *A. precatorius* L. was given at doses of 1, 2, 5, 10 and 30 mg/kg to different groups of mice each group containing six animals. Watery suspension of the test drug was given to the animals orally through a feeding tube. After administering the test drug, the animals were observed for the first three hours for any toxic symptoms followed by observation at regular intervals for 24 hours up to seven days. At the end of the study, the animals were also observed for general organ toxicity, morphological behavior and and mortality.

**Chemicals**

All chemicals used in this study were purchased from Sigma Chemical Company, Mumbai. Chemicals were of analytical grade with high purity.

**Experimental design**

Leaves of *A. precatorius* L. were properly washed, shade dried and powdered. 100g of this powder were separately extracted in five sets of experiments with a) 1000 ml of 10 : 1 (v/v) ethanol – chloroform mixture.
b) 1000 ml of 10 : 1 (v/v) methanol – chloroform mixture.
c) 1000 ml of 10 : 1 (v/v) methanol – chloroform mixture.
d) 1000 ml of 10 : 1 (v/v) acetone – chloroform mixture.
e) 1000 ml of 10 : 1 (v/v) acetone – petroleum ether – chloroform mixture.

Extraction in each case was for ½ h on a rotary shaker. It was then centrifuged. Supernatant was collected and evaporated to dryness. Dry brown mass was obtained.

Rats were divided into two groups of 20 each. First group of animals took normal diet while animals of the second group, in addition to normal diet, took isolated dry brown mass obtained after solvent extraction in the dose of 0.5g/kg body weight daily through oral route. Experiment was continued for 40 days. Separate rats were used for different solvent extraction groups.

**Growth of rats**

Growth of rats was measured on 10th, 20th, 30th and 40th day. Overall behavior of the animals was noted.

**Statistical analysis**

The values were expressed as mean ± SEM and were analyzed using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS). Differences between means were tested employing Duncan’s multiple comparison test and significance was set at p < 0.05.

**RESULTS**

**Acute toxicity studies**

Acute toxicity studies revealed that leaves of *Abras precatorius* L. did not produce any toxic symptoms when administered orally to mice in doses of 1, 2, 5, 10 and 30 mg/kg. Animals were healthy, cheerful and behaved normal throughout the experimental period. No death of animal was recorded during seven days of experiment.

Table – 1 shows effect of isolated brown mass after extraction of *Abras precatorius* L. leaves powder with 1000 ml of 10 : 1 (v/v) ethanol – chloroform mixture on body weight of rats. It appears from the table that the isolated brown mass from *A. precatorius* L. could not decrease body weight of rats on 40th day of experiment.

Table – 2 shows effect of isolated brown mass after extraction of *Abras precatorius* L. leaves powder with 1000 ml of 10 : 1 (v/v) methanol – chloroform mixture on body weight of rats. It appears from the table that the isolated brown mass from *A. precatorius* L. could not decrease body weight of rats on 40th day of experiment.

Table – 3 shows effect of isolated brown mass after extraction of *Abras precatorius* L. leaves powder with 1000 ml of 10 : 1 (v/v) acetone – chloroform mixture on body weight of rats. It appears from the table that the isolated brown mass from *A. precatorius* L. could decrease body weight of rats from 20th day up 40 days of experiment and the results were statistically significant up to the level p < 0.001 when compared with the control group.

Table – 4 shows effect of isolated brown mass after extraction of *Abras precatorius* L. leaves powder with 1000 ml of 10: 1 (v/v) petroleum ether – chloroform mixture.
mixture on body weight of rats. It appears from the table that the isolated brown mass from *A. precatorius* L. could not decrease body weight of rats after on 40\(^{th}\) days of experiment.

Table – 5 shows effect of isolated brown mass after extraction of *A. precatorius* L. leaves powder with 1000 ml of 10 : 1 (v/v) acetone – petroleum ether mixture on body weight of rats. It appears from the table that the isolated brown mass from *A. precatorius* L. could decrease body weight of rats from 20\(^{th}\) day up 40 days of experiment but the results were not statistically significant when compared with the control group.

**Table 1.** Showing effect of isolated brown mass after extraction of *Abrus precatorius* Linnaeus leaves powder with 1000 ml of 10 : 1 (v/v) ethanol – chloroform mixture on body weight of rats. (Changes of body weight in gram)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>10(^{th}) day</th>
<th>20(^{th}) day</th>
<th>30(^{th}) day</th>
<th>40(^{th}) day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>40.9 ± 2.3</td>
<td>58.2 ± 2.1</td>
<td>62.9 ± 2.3</td>
<td>70.2 ± 2.2</td>
</tr>
<tr>
<td>2</td>
<td>Isolated brown mass from <em>A. precatorius</em> L. leaves</td>
<td>38.8 ± 1.9</td>
<td>55.7 ± 1.7</td>
<td>60.7 ± 1.5</td>
<td>65.3 ± 1.8</td>
</tr>
</tbody>
</table>

**Table 2.** Showing effect of isolated brown mass after extraction of *Abrus precatorius* Linnaeus leaves powder with 1000 ml of 10 : 1 (v/v) methanol – chloroform mixture on body weight of rats. (Changes of body weight in gram)

<table>
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<th>40(^{th}) day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>38.3 ± 2.0</td>
<td>59.8 ± 2.5</td>
<td>60.6 ± 2.1</td>
<td>68.8 ± 2.1</td>
</tr>
<tr>
<td>2</td>
<td>Isolated brown mass from <em>A. precatorius</em> L. leaves</td>
<td>35.1 ± 1.7</td>
<td>56.5 ± 1.8</td>
<td>57.1 ± 1.5</td>
<td>66.9 ± 1.7</td>
</tr>
</tbody>
</table>

**Table 3.** Showing effect of isolated brown mass after extraction of *Abrus precatorius* Linnaeus leaves powder with 1000 ml of 10 : 1 (v/v) acetone – chloroform mixture on body weight of rats. (Changes of body weight in gram)

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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>41.5 ± 1.9</td>
<td>58.9 ± 1.3</td>
<td>61.6 ± 1.6</td>
<td>70.7 ± 2.0</td>
</tr>
<tr>
<td>2</td>
<td>Isolated brown mass from <em>A. precatorius</em> L. leaves</td>
<td>39.7 ± 1.2</td>
<td>50.7 ± 1.6*</td>
<td>52.3 ± 1.4*</td>
<td>55.2 ± 1.6**</td>
</tr>
</tbody>
</table>

*p<0.01, ** p< 0.001

**Table 4.** Showing effect of isolated brown mass after extraction of *Abrus precatorius* Linnaeus leaves powder with 1000 ml of 10 : 1 (v/v) petroleum ether – chloroform mixture on body weight of rats. (Changes of body weight in gram)

<table>
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</tr>
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<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>38.6 ± 1.0</td>
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<td>60.9 ± 1.9</td>
<td>67.9 ± 2.2</td>
</tr>
<tr>
<td>2</td>
<td>Isolated brown mass from <em>A. precatorius</em> L. leaves</td>
<td>37.1 ± 1.1</td>
<td>56.9 ± 1.3</td>
<td>58.7 ± 1.7</td>
<td>66.7 ± 1.5</td>
</tr>
</tbody>
</table>

**Table 5.** Showing effect of isolated brown mass after extraction of *Abrus precatorius* Linnaeus leaves powder with 1000 ml of 10 : 1 (v/v) acetone – petroleum ether mixture on body weight of rats. (Changes of body weight in gram)

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<tr>
<td>1</td>
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**Fig 1.** *Abrus precatorius* Linnaeus.
DISCUSSION

_ Abrus precatorius _ Linnaeus is a medicinal plant. Since Vaidic period _ A. precatorius _ L. has been used for therapeutic purpose (Gurung Bejoy, 2002). The plant is popularly known as Gunja, Rosary pea, jequirity bean etc. It belongs to the family leguminosae (Fabaceae). The plant is found through out India in hedges and bushes in exposed areas.

Roots, seeds and leaves of _ A. precatorius _ L. are used in traditional Medicine. The plant is mainly used in the treatment of ulcer and skin infection (Chopra et al., 1958). Seeds of the plant are very much attractive but are deadly poisonous. They are used in ornaments. Pharmacological studies revealed that seeds have anti diabetic property and can induce abortion (Monago CC et al., 2005; Noumi Emmanuel and Djeumen, 2007). Seeds also have anti oxidative property as well as anti inflammatory analgesic activity (Pal Ranju S et al., 2009; Arora Rashmi, Gill Singh Naresh et al., 2011; Anbu J, Ravichandiran V et al., 2012).

Much work has been done on antimicrobial activity of the aqueous extract of _ A. precatorius _ L. It was found out that the plant could exert antimicrobial effect against _ Klebsiella pneumoniae_, _ Streptococcus pyogenes_, _ Salmonella typhimurium_, _ Escherichia coli_, and _ Streptococcus pneumonia _ (Saganuwan SA et al., 2005; ). The plant is also found efficacious in cancer (Ravichandiran V et al., 2012) and in malaria.

A wide range of active components including glycoside abralin, an albuminous substance ‘abrin’, abrasine, abrugenic acid-methylester, abruslectone, abrussic acid, anthocyanins etc. have been isolated from the plant (Duke JA et al., 2002; Kishor S et al., 2012).

Recently we found that plant _ A. precatorius _ L. could exert body weight loss in albino rats. Results are under communication. We intended to isolate the active compound responsible for the said activity. In connection with the isolation work we studied effects of different solvent systems to obtain active fraction. Five solvent systems were used. They were, 10 : 1 (v/v) ethanol – chloroform mixture, 10 : 1 (v/v) methanol – chloroform mixture, 10 : 1 (v/v) acetone – chloroform mixture, 10 : 1 (v/v) petroleum ether – chloroform mixture and 10 : 1 (v/v) acetone – petroleum ether mixture.

**CONCLUSION**

Effect of solvent on extracted mass from leaves of _ A. precatorius _ L. on body weight of rats was studied. Results showed that mass obtained after extraction with 10 : 1 (v/v) acetone – chloroform mixture had maximum body weight reduction activity in albino rats (Figure 2).

**REFERENCES**


