Documentation of ethno-botanicals and evaluation for their anthelmintic activity in Cholistan, Pakistan

Muhammad Asif Raza
(PhD Scholar)

Supervisors:
Prof Dr. Eva Schlecht
Prof Dr. Muhammad Younas

ICDD, Graduate School of Socio-Ecological Research for Development
University of Kassel, Germany
June 28, 2010
Outline

1. Introduction
2. Problem statement
3. Materials & Methods
4. Statistical analysis
5. Time plan
6. Possible outcomes
1. Introduction

• In Pakistan almost 2000 medicinal plant species exist but very few exploited
• Country’s medicinal herbs requirement (90%) is met through import
• About 50% of the population cured using traditional medicines
• More than 40,000 traditional herbal practitioners (Anonymous, 1999-2002)
• Problems due to chemotherapeutic control practices are,
  • Side effects
  • Resistance development
  • Chemical residues
  • Toxicity problems
  • Un-economical
  • Non-adaptability of drugs
  • Non-availbity in remote areas

• These considerations have revived interest in exploiting the potential of medicinal plant drugs which could be safer & not expected to produce residue problems.
2. Problem Statement

• In Pakistan parasitism is one of the major menace for livestock, causing obstacles in the development of profitable livestock industry (Khan et al., 1989; Sajid et al., 1999)

• Prevalence of helminths in ruminants 25-92% in different areas of Pakistan (Iqbal et al., 1993; Raza et al., 2007).
• Synthetic anthelmintics are
  • Expensive
  • Unavailable to farmers in rural areas
  • Drug resistance
  • Food residues
  • Environmental pollution
Possible Solution

- Pakistan has large list of medicinal plants but not using these

- Need screening the medicinal plants for their anthelmintic activity
Study Site

- Cholistan (*Rohi*) spreads in 3 districts of Punjab viz; Bahawalpur, Bahawalnagar and RY Khan & covers an area of 66,55,360 acres (10399 Sq. miles)
  - Temperature 6-50 °C
  - Rainfall 128-175 mm
  - Ground water mostly brackish, 25-90 m deep
  - Sweet water zones hakra bed, canal seepage zones
  - Human population 0.155 million
  - Livestock population 1.6 million
• Livestock husbandry is very important in the community & traditionally wealth is being assessed based on the number of livestock.

• Plants of this desert have great ethno-botanical importance.

• A little work has been done
Map of Study Area
3. Materials and Methods

3.1. Base-line survey for the documentation of medicinal plants

3.2. Prevalence of Helminthes

3.3. Evaluation of anthelmintic activity
3.1. Baseline survey for the documentation of medicinal plants

- A well-structured questionnaire to interview 100 stockholders/ farmers and 20 local healers.

- Areas/ villages selected for baseline survey
  1. 148 DB
  2. 183 DB
  3. 423 DB
  4. 335 HR
  5. 123 DNB

- Documented plants will collect & identify by Botanist.
Some Medicinal Plants

*Ficus religiosa L.* (Pipal)

*Calotropis procera (L)* (Aak)

*Convolvulus arvensis* (One wehri)
3.2. Prevalence of helminthes

**Sample Collection**
- Faecal samples (200) of sheep & goat will collect in sterile polythene bags directly from rectum of each animal

**Faecal Examination**
- Direct technique
- Indirect technique (Floatation technique)
Direct technique

- 1 g faecal sample mix well in a drop of water
- Examine under microscope by placing a drop of suspension on slide with cover slip
- At least 3 direct smears should examine from each sample
Indirect technique

- 5 g faeces mix in 30-50 ml water
- Sieve to remove course material
- Allowed to sediment for half an hour
- Pour off supernatant, mix sediment in saturated NaCl solution
- Centrifuge at 1000 rpm for 2 minutes
- Upper 0.1 ml suspension transfer to a glass slide
- Examine under microscope at 10 X for the presence of helminth eggs
3.3. Evaluation of anthelmintic activity

**Collection of Plant materials**

- Plant materials will be collected from Cholistan desert
- Sample will be dried at about 50-52°C
- 500 g of the each plant material will be ground first to pass a 2 mm screen
Methanolic extract preparation

- Plant material dried in shade
- Ground to powder in an electric mill,
- Stored in cellophane bags at 4°C.
- Powdered plant extracted with Methanol in a Soxhlet’s apparatus (Asuzu and Onu, 1994)
- Crude methanolic extract (CME) stored at 4°C until used.
In *vitro* anthelmintic activity

**Two techniques**

- Egg hatch test
- Larval development test
How to recover eggs of helminthes?

- Mix 50 g faeces in 50 ml water with electric mixer
- Sieve & mix 100 ml saturated NaCl solution
- Pour into shallow tray having 4 cm depth
- Place a plastic sheet on mixture
- Egg adhere to floating plastic sheet due to less specific gravity
- Remove it after 15 minutes & wash with water to collect eggs
- Number of eggs will be estimated by McMaster technique (Soulsby, 1982)
**Egg hatch test** (Coles *et al.* 1992)

- 0.2 ml suspension containing eggs will be distributed in a 24-flat-bottomed microtitre plate
- Mix with different concentrations of plant extract i.e., CME.
- Control plates will contain the water
- Eggs incubated in this mixture for 48 h at 25°C
- One drop of Lugol’s iodine solution will be added
- Eggs and first-stage larvae (L1) in each plate will be counted
Larval development test (Ademola et al., 2004)

- In a test tube add 150 µl of nutritive medium (Hubert and Kerboeuf, 1992) to 500 µl of egg suspension containing approximately 100 eggs
- Cover & place it in an incubator at 25°C for hatching of the eggs to L1 in 48 h
- Add CME at different concentrations to L1
- After 7 days, larvae will be counted as living and dead third stage larvae (L3)
All data collected will be analyzed with appropriate statistical method
## 5. Time plan

<table>
<thead>
<tr>
<th>Activity</th>
<th>Time needed</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation</td>
<td>2 ½ months</td>
<td>Literature collection, secondary data collection</td>
</tr>
<tr>
<td>GPS training</td>
<td>15 days</td>
<td>GPS training University of Kassel, Germany (April 2010)</td>
</tr>
<tr>
<td>Field work</td>
<td>8 months</td>
<td>Base-line survey, collection of plants and prevalence of helminthes</td>
</tr>
<tr>
<td>Laboratory work</td>
<td>8 months</td>
<td>Preparation of plant extracts and in vitro evaluation.</td>
</tr>
<tr>
<td>Data evaluation and publication</td>
<td>1 year</td>
<td>Statistical analyses of data, evaluation, publications and thesis write up</td>
</tr>
</tbody>
</table>
6. Possible Outcomes

• Natural resources in the form of plants can be utilized for the treatment of diseases

• Ethno-botanicals are economical, easily available and helpful for poor peoples of Rohi

• Treatment of helminths instead of purchasing/importing costly anthelmintic medicines can be possible

• Help in preserving the natural plant fauna of Cholistan

• Provoke further pharmacological and phytochemical research on medicinal plants
Thank you