

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

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# 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





## 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



## Contd..

### 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)



# Contd..

## 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)



# Contd..

## 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



# Contd..

## Indirect technique

- 5 g faeces mix in 30-50 ml water
- Sieve to remove coarse 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





## Contd..

### 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



# Contd..

## 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.



# Contd..

In *vitro* anthelmintic activity

## Two techniques

- Egg hatch test
- Larval development test



# Contd..

## 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)



# Contd..

## 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



# Contd..

## Larval development test (Ademola *et al.*, 2004)

- In a test tube add 150  $\mu$ l of nutritive medium (Hubert and Kerboeuf, 1992) to 500  $\mu$ l 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)



## 4. Statistical Analyses

All data collected will be analyzed with appropriate statistical method



## 5. Time plan

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





## 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*

