

**ANTHELMINTIC ACTIVITY OF HORSE RADISH****Mathews Abraham<sup>1\*</sup>, Anjana abraham<sup>2</sup>, C. R. Biju<sup>3</sup>, G. Babu<sup>4</sup>**

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**ABSTRACT**

Helminth infections are among the most common infections in humans, affecting a large population of the World. Use of herbal products as antimicrobial agents may provide the best alternative to the wide and injudicious use of synthetic antibiotics. Albendazole, the commercial anthelmintic drug is used as a standard reference and saline as control. The predominant effect of anthelmintic drug on the worms is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Albendazole acts probably by blocking glucose uptake and depletion of its glycogen stores.

**KEYWORDS:** Anthelmintic activity, Horse radish, Albendazole.

**INTRODUCTION**

Helminthic infections are the major health problem in tropical countries and many people are suffering from worm infestations. Helminths (Worms) can cause various gastrointestinal and general symptoms. They also cause blood loss, nutritional deficiencies, urticaria allergic manifestations and even intestinal obstruction. Humans are the primary hosts for many helminthic infections. Most of the worms reproduce sexually in human host, producing egg and larvae which pass out of the body and infect secondary or intermediate host. Helminthic infections are rarely fatal, but are major cause for ill health. Helminths mainly cause infection by living in host's alimentary canal or in other tissues of the host's body.

The main worms that live in the host's alimentary canal are

1. Tapeworms (Cestodes)
2. Intestinal round worms (Nematodes)

The main worm that live in the tissue of the host are

1. Trematodes or Flukes
2. Tissue round worms
3. Hydatid tape worm

Horseradish is a (*Armoracia rusticana*, syn. *Cochlearia armoracia*) is a perennial plant of the Brassicaceae family (which also includes mustard, wasabi, broccoli and cabbage) the plant is probably native to southeastern Europe and western Asia. It is now popular around the world. It grows up to 1.5 meters (5 feet) tall, and is cultivated primarily for its large, white, tapered root. The intact horseradish root has hardly any aroma. When cut or grated, however, enzymes from the now-broken plant cells break down sinigrin (a glucosinolate) to produce allyl isothiocyanate (mustard oil), which irritates the mucus membrane of the sinuses and eyes. Once exposed to air (via grating) or heat, if not used immediately or mixed in vinegar, the grated mash darkens, loses its pungency, and becomes unpleasantly bitter-tasting.

## **MATERIALS AND METHODS**

### **Collection of plant and earth worm**

The fully developed plant Horse Radish collected from ooty

The Indian adult earth worms were used to study the anthelmintic activity. The earth worms collected from the swamp areas and washed with normal saline to remove all the mud and fecal matter. The adult earth worm of 7-9 cm and 0.2-0.4cm in width were used for investigation.

### **Authentication**

The collected plant was identified and confirmed by botanical department, university of Calicut.

### **Microscopy**

Microscopic studied of both leaf and rhizome was carried out. For that t.s of both leaf and rhizome was taken and studied.

### **Preparation of plant extract**

250gm of powdered rhizome and dried leaf of Horse Radish was extracted with organic solvent (ethanol) and water (aqueous). The alcoholic extract was done by using soxhelt apparatus.

Preparation of aqueous leaf extract.

Preparation of aqueous rhizome extract.

Preparation of alcoholic leaf extract.

Preparation of alcoholic rhizome extract.

### **Soxhelation**

Also known as continuous extraction. This is modification of simple percolation method where in percolation is affected by the use of a soxhelt extractor where by a small volume of hot liquid is made to percolate through a column of drug again and again by evaporation and subsequent condensation. A soxhelt extractor consist of a flask holding the menstruum, the extractor or a cylindrical percolator provided with an attached siphon called the soxhelt extractor and a reflux condenser fitted at top of soxhelt. The material to be extracted is usually placed in thimble made of filter paper and then inserted in to the extractor. The menstruum is placed in the flask and boiled.

The vapors arising from the flask pass by the side tube in to condenser. The vapors is condensed and drips in to the body of the extractor as pure menstruum. It percolates through the drug to be extracted and dissolving the soluble substances.

As soon as the level of the menstruum in the extractor rises above the siphon bend, the extract is drained out and flowing through the siphon in to the flask. This alternate filling and emptying of the body of the extractor goes on continuously and in the cycle the distillate obtained from the extract(pure menstruum) gets delivered up on the drug, extracts its soluble constituents and flows in to the flask where it is evaporated leaving behind the extract matter. Thus the same quantity of the menstruum is recycled every time and complete extraction is achieved with a very small volume of the menstruum.

### **PHYTOCHEMICAL SCREENING OF EXTRACT**

The compound that was responsible for the medicinal property of a drug is usually secondary metabolites. A systemic study of crude drug embraces through consideration of primary and secondary metabolites derived as a result of plant metabolism. The detection of these various constituents is carried out by photochemical screening techniques.

**PRELIMINARY PHYTOCHEMICAL ANALYSIS****CARBOHYDRATES**

Small quantities of alcoholic extract was dissolved separately in 5ml of distilled water and filtered. This filtrate was subjected to various tests to detect the presence of carbohydrates.

**1. Molisch's tests**

2-3ml of alcoholic extract was added with few drops of naphthol solution and added concentrated sulphuric acid from the sides of the test tubes, violet ring is formed at the junction of two liquids.

**2. Fehling's test**

Mixed 1ml of Fehling's solution a&b then boiled for 1min, followed by addition of an equal volume of test solution, the content in a bath for 5-10 min, first yellow then brick red precipitate was observed.

**3. Benedict's test**

Mixed equal volume of Benedict's reagent and the test solution was taken in a test tube. Heated in boiling water bath for 5min, solution appeared as green, yellow or red depending upon the amount of reducing sugar present in the test solution.

**FIXED OILS AND FATS**

Fixed oils and fats are confirmed by chemical test for glycerin, which is produced by their hydrolysis.

1. Treat 5 drops of sample with 1ml of 1% of copper sulphate solution. Then add 10% NaOH solution. A clear blue solution is obtained, which shows glycerin is present in the sample.

**2. Saponification test**

Add few drops of alcoholic potassium hydroxide to test solution along with a drop of phenolphthalein separately and heat on water bath for 1-2 hr. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

**PROTEINS AND AMINOACIDS**

1. **Biuret test:** Few amount of the extract was added to 4% NaOH solution and along with a few drops of copper sulphate, the development of violet to pink colour indicates the presence of proteins.

## 2. Xanthoprotein test

A small amount of sample warmed with concentrated nitric acid formed yellow colour. The colour turned orange when solution was made alkaline. The colour is due to the nitration of aromatic ring present in phenyl alanine, tyrosine and tryptophan.

## 3. Heat test

Heat the test solution in boiling water bath proteins get precipitated.

## ALKALOIDS

A small amount of alcohol extract was taken in a test tube, and added with 5ml of 1.5ml HCl and then filtered. The filtrate was used for presence of alkaloids by the following test.

### 1. Dragondroff's reagent

Alcoholic extract is treated with dragondroff's reagent, reddish brown precipitate is formed.

### 2. Mayer's reagent

Small amount of alcoholic extract is treated with Mayer's reagent, cream colour precipitate is formed.

### 3. Hager's reagent

Alcoholic extract is treated with Hager's reagent, yellow colour precipitate was formed.

## TANNINS AND PHENOLIC COMPOUND

2-3ml of or alcoholic extract was taken and added with a few drops of following reagent in separate test tubes.

5% ferric chloride solution	: deep blue-black colour
Lead acetate solution	: white precipitate
Gelatin solution	: white precipitate
Bromine water	: discoloration
Acetic acid solution	: red colour solution

## PHYTOSTEROLS

### 1. Salkowski reaction

2ml of extract was added with 2ml methyl chloride and concentrated sulphuric acid then shaken well. Methyl chloride layer showed red and acid layer shown greenish yellow fluorescence.

## 2. Liber mann burchard reaction

2ml of extract were mixed with methyl chloride. 1-2ml acetic anhydride and 2 Drops of concentrated sulphuric acid was added from the sides of test tube, initially red then blue and finally green colour was observed.

## TERPENOIDS

### 1. Terpenoids salkowski reaction

Small amount of extract treated with concentrated sulphuric acid, it gives red colour at lower layer, it indicates presence of steroids and formation of yellow colour at lower layer, and it indicates the presence of terpenoids.

### 2. Liber mann burchard reaction

Small amount of extract treated with acetic anhydride, boil and cool. Then concentrated sulphuric acid added through sides of test tube, brown ring colour obtained at the junction of two layer and upper layer turns green which shows the presence of steroids and formation of deep red colour indicates the presence of triterpenoids.

## RESULT AND DISCUSSION

From qualitative chemical evaluation of the extract of the plant Horse Radish, it was revealed that the extract showed the presence of carbohydrates, fixed oils, alkaloids, volatile oils, tannins, glycosides and flavanoids.

The predominant effect of anthelmintic drug on the worms is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Albendazole acts probably by blocking glucose uptake and depletion of its glycogen stores. Intra cellular microtubules in the cells of the worm gradually lost. The extract of Horse Radish not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 20% mg/ml, in shorter time as compared to reference.

## CONCLUSION

From the above result, it is concluded that Horse Radish used by tribal traditional to treat intestinal worm infection, showed significant anthelmintic activity. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plant as anthelmintic. These promising result show the scope for further studies to isolate

and reveal the active compound contained in the crude extracts of Horse Radish responsible for anthelmintic activity.

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