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PHYTOCHEMICAL SCREENING AND ANTHELMINTIC ACTIVITY OF *CATHARANTHUS ROSEUS* EXTRACT USING COMBINATION OF SOLVENTS AGAINST *PHERETIMA POSTHUMA*

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Abstract

Helminthiasis is also known as worm infection, is macro parasitic disease of humans and other animals in which a part of the body is infected with parasitic worms, known as helminthes. Helminthiasis have been targeted under the joint action of the world's leading pharmaceutical companies and non-governmental organizations through a project launched in 2012 called the London Declaration on Neglected Tropical Diseases, which aims to control or eradicate certain neglected tropical diseases by 2020.

Catharanthus roseus, also known as Madagascar periwinkle or vinca rosea is a perennial shrub. It is revealed that this plant have anthelmintic activity, but so far no work has been carried out using combinations of solvents for leaves of *C. roseus* for carrying out anthelmintic activity.

In-vitro anthelmintic activity carried out against Indian adult earthworm *Pheretima posthuma* using dichloromethane: methanol leaves extract of *Catharanthus roseus* showed significantly good activity when compared to standard albendazole and increases with increase in concentration.

Key words: Helminthiasis, *Catharanthus roseus, Pheretima posthuma* and anthelmintic activity

Introduction

Helminthiasis is also known as worm infection, is macroparasitic disease of humans and other animals in which a part of the body is infected with parasitic worms, known as helminths. There are numerous species of these parasites, which are broadly classified into tapeworms, flukes, and roundworms. They often live in the gastrointestinal tract of their hosts, but they may also burrow into other organs, where they induce physiological

damage[1]. Soil-transmitted helminthiasis and schistosomiasis are the most important helminthiasis, and are among the neglected tropical diseases.This group of helminthiasis have been targeted under the joint action of the world's leading pharmaceutical companies and non-governmental organizations through a project launched in 2012 called the London Declaration on Neglected Tropical Diseases, which aims to control or eradicate certain neglected tropical diseases by 2020.[1]

Anthelminthic are a group of antiparasitic drugs that expel parasitic worms (helminths) and other internal parasites from the body by either stunning or killing them and without causing significant damage to the host. There are a number of herbs which are known to have anthelmintic properties (e.g. wormwood). Numerous plants are being tested for their anthelmintic properties (e.g. pumpkin seed, garlic). So far, none have been proven under formal research conditions to be effective anthelmintic.[2]

Materials and methods

Collection of plant material:

The basic plant material of *Catharanthus roseus* was collected from medicinal garden of Gyana Jyoti College of Pharmascy, Nalgonda, and Telangana, India. The plant was identified and authenticated by Department of Botany, Osmania University, Hyderabad, Telangana, India (voucher number 0359).

Preparations of Extract: Fresh leaves of *catharanthus roseus* were collected washed thoroughly in tap water to remove traces of soil and other contaminants and then with distilled water. It was then shade dried. Further the dried material is subjected to chopping and electric grinding for obtaining coarse powder. About 60gms of powdered material was subjected to extraction with dichloromethane: methanol (1:1) using soxhlet apparatus for 12 hrs. The extract was concentrated to obtain the dried form. The extract yield obtained was about 12gm.

Phytochemical Screening[3-6]

The extract was subjected to preliminary phytochemical screeining for the presence of Alkaloids, Glycosides, Carbohydrates, Flavonoids, Phenols, Tannins, Terpenes, proteins and amino acids and fats and oils.

- **Detection of alkaloids:**

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) *Mayer's Test:*

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

b) *Wagner's Test:*

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c) *Dragendroff's Test:*

Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d) *Hager's Test:*

Filtrates were treated with Hager's reagent (saturated picric acid solution).Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

- **Detection of carbohydrates:**

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) *Molisch's Test:*

Filtrates were treated with 2 drops of alcoholic α-Naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates**.**

b) *Benedict's Test***:**

Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

c) *Fehling's Test:*

Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

- **Detection of glycosides:**

Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) *Modified Borntrager's Test:*

Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

b) *Legal's Test:*

Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

- **Detection of saponins**

a) *Froth Test:*

Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

b) *Foam Test:*

0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

- **Detection of phytosterols**

a) *Salkowski's Test:* Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

b) *Libermann Burchard's test:*

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

- **Detection of phenols**

Ferric Chloride Test:

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

- **Detection of tannins**

Gelatin Test:

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

- **Detection of flavonoids**

a) *Alkaline Reagent Test:*

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

b) *Lead acetate Test:*

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

- **Detection of proteins and aminoacids**

a) *Xanthoproteic Test:*

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

b) *Ninhydrin Test:*

To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

- **Detection of diterpenes**

Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Anthelmintic activity:[7-10]

Indian adult earthworms (*Pheretima posthuma*) were collected from moist soil of a farm house in Moinabad, Ranga Reddy dist. The earthworms were maintained under normal vermicomposting medium with adequate supply of nourishment and water. Before the initiation of experiment, the earthworms were washed with normal saline. Adult earthworms of approximately 8-10 cm in length and 0.2-0.3 cm in width were used for the experiment.These

worms was the selected model for anthelmintic activity due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings.

Animal grouping: Group I: Control (20 ml of normal saline solution).

Group II: Standard (Albendazole 20 mg/ml)

Group III: Leaves extract 50 mg/ml

Group IV: Leaves extract 100 mg/ml

Group V: Leaves extract 150 mg/ml

Preparation of Normal saline solution: 9 gm of sodium chloride was dissolved in 1000 ml of distilled water in a volumetric flask and volume is made up to the mark with distilled water.

Preparation of standard solution (20 mg/ml):

The weighed amount of powdered albendazole was dissolved in small amount of DMSO and then made up to 20ml using normal saline solution.

Preparation of extract solution:

The required amounts of dichloromethane: methanol leaves extract of *catharanthus roseus* were weighed for 50 mg/ml, 100 mg/ml and 150 mg/ml solutions and were dissolved separately in small amount of DMSO and then the volume was made to 20 ml using normal saline solution separately.

Procedure:

The anthelmintic activity on leaves extract *Catharanthus roseus* was evaluated as per the method reported by Dash *et al* on adult Indian earthworm *Pheretima posthuma*. 20 ml of sample solution containing three different concentrations of dichloromethane: methanol (1:1) extract (50, 100 and 150mg/ml) and standard albendazole solution (20mg/ml) were prepared and kept in separate Petri plates. Approximately equal size three earthworms were released in each group. Observations were made for the time taken for paralysis and death of individual worms. Paralysis is said to occur when the worms do not revive even in normal saline and death was concluded when the worms lose their motility and do not revive in warm water and with the fading of color. 20 ml normal saline was used as control group. Time was noted in minutes for all worms individually.

Figure 1: Earth worms in control group.

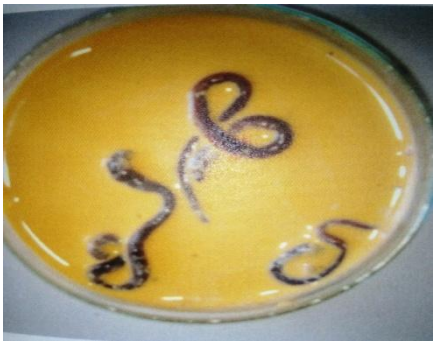

Figure 2: Earth worm in standard Albendazole.

Figure 3: Earth worms in Leaves extract of *Catharanthus roseus.*

Results and discussion:

Phytochemical Investigation:

The preliminary phytochemical analysis of Methanol leaves extract of *Piper Betel* revealed the presence of alkaloids.

SL No.	Test	DME
	Carbohydrates	+
	Alkaloids	+
	Glycosides	+
	Saponins	-
	Phytosterols	
	Phenols	+
	Tannins	-
	Flavonoids	-

	Proteins and aminoacids	-
	Terpenes	-

DME: dichloromethane: methanol leaves extract

Anthelmintic activity:

In in-vitro study, it is found that dichloromethane: methanol leaves extract of *catharanthus roseus* exert anthelmintic activity against Indian adult earth worm *Pheretima posthuma* in the concentration range of 50-150 mg/ml and showed increase inactivity with increase in concentration (dose dependent). As shown in Table-1and chart-1, the extract showed significantly more activity with 150mg/ml with paralytic time of 17.08 min and death time of 19.34 min when compared to standard with paralytic time of 35.33 min and death time of 60.33 min. Dichloromethane: methanol leaves extract of 50mg/ml and 100mg/ml also showed anthelmintic activity with the paralysis time 55.33 and 50.07 min respectively and death time of 70.07and 62.12 min respectively but to a lesser extent when compared to standard albendazole.

Table 1: Paralysis and death time of leaves extract of *catharanthus roseus* against *Pheretima posthuma.*

S No.	Group	Paralysis time(min)	Death time(min)
1	I	-	-
2	II	35.33±4.37	60.33±1.67
3	III	55.33±11.74	70.07±1.35
4	IV	50.07±3.75	62.12±1.25
5	V	17.08±0.27	19.34±0.00

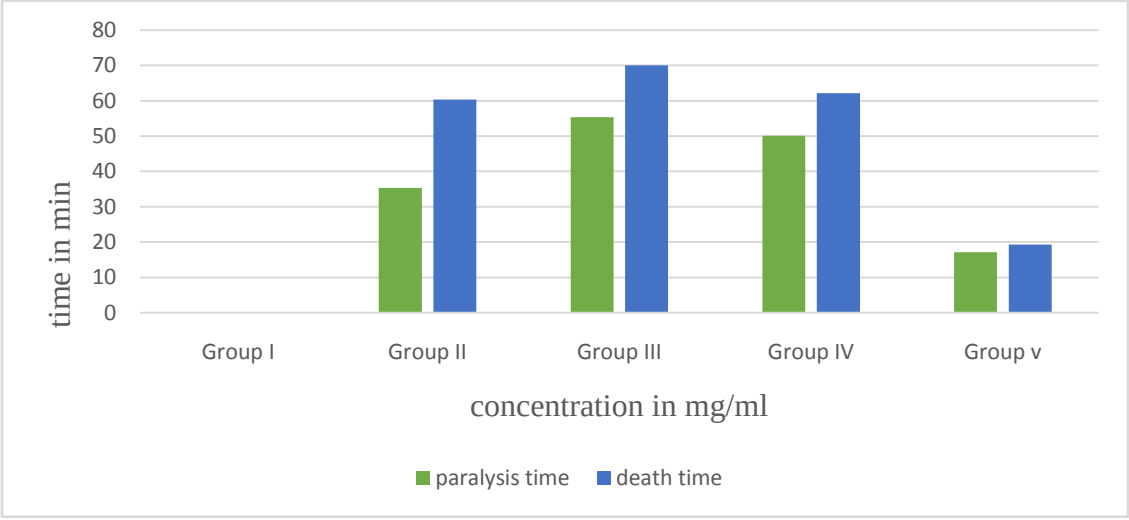

Chart1: Chart showing paralysis time and death time of leaves extract of *Catharanthus roseus* against *Pheretima posthuma.*

Conclusion

The present study showed that, *Catharanthus roseus* leaves dichloromethane: methanol extract is found to exhibit a significant anthelmintic activity against *Pheretima posthuma*. Therefore, further work can be carried out for the isolation and molecular characterization of active constituents responsible for anthelmintic activity.

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