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Phytochemical Screening and In-Vitro Anthelmintic Activity of *Pithecellobium dulce* Leaves

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ABSTRACT

The present research work is about the phytochemical investigation and anthelmintic activity of *Pithecellobium dulce* leaves. Standardization of crude drugs extracted from plants plays an important role in identifying the quality and purity of drugs. Phytochemical constituents are extracted by petroleum ether, chloroform, and methanol from *Pithecellobium dulce* leaves belong to the family *Leguminosae*. This research finds highlights that methanolic extracts of *Pithecellobium dulce* leaves had the highest number of phytochemicals compared to other solvent extracts. Hence, methanolic extracts of *Pithecellobium dulce* leaves hold the greatest potential to treat various human diseases and have profound medical applicability. In-vitro anthelmintic activity was evaluated by taking adult Indian earthworms, *Pheretima posthuma* having anatomical and physiological resemblance with intestinal roundworms. The earthworms were washed in normal saline solution before they were placed into 10ml of the respective formulation. To observe anthelmintic activity, all the investigations were carried out by methanolic extract with different concentrations of 10, 20, 50mg/ml, significant activity like time of paralysis and time of death were noted. At the highest concentrations of 50mg/ml, significant anthelmintic activity was observed and compared with piperazine citrate (10, 20, 50mg/ml as standard reference and distilled water as control. Herbal drugs and synthetic drugs were equally effective in helminthic infestations but a methanolic extract of Indian medicinal plants exhibits potentiality and has maximum anthelmintic activity.

INTRODUCTION

The use of medicinal products and supplements has increased exceedingly over the past decades, not less than 80% of the world population depends on medicinal plant products for their primary health. In many developing countries, major proportion of the population are depending on traditional practitioners and using medicinal plant products to get their health in good conditions [1]. *Pithecellobium dulce* Benth belongs to the family *Leguminosae*, which is widely distributed in the greater part of India and is also found in Southeast Asia. In recent years, nutraceuticals have acquired huge attention as nutritional supplements for their positive physiological effects in human beings [2], from villages to developed cities, traditional ways of natural medication consecutively becoming popular. Afzelin (kaempferol-3-O-α-L-rhamopyranoside) was isolated from *Pithecellobium dulce* leave which has antimycobacterial property. The leaves are also reported to have anti-inflammatory, analgesic, antioxidant, and antidiabetic activities [3-5]. The *Pithecellobium dulce* fruits have also been studied for anti-inflammatory activity due to saponin content, free radical scavenging, and gastro-protective, antidiabetic, and hepatoprotective effects [6-9]. Various phytochemical constituents with medicinal properties to cure various health illnesses have been revealed every day by researchers [10-14]. Parasitic infections are common in tropical regions that infect humans. Parasitic diseases disable their hosts and render them incapable of leading a normal life and in certain cases, they cause mortality of the affected human hosts. Bioactive plant metabolites are cheap, cost-effective, and easily affordable drugs against parasitic infections. Helminthiasis is one of the most common diseases that widespread to human beings and animals due to poor management. To treat parasitic infections in man and animals, a large number of medicinal plants are being used. The assay was carried out on an adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings [10,11]. Because of easy availability, *Pheretima posthuma* was used for the evaluation of anthelmintic activity in vitro, the present research work deals with the evaluation of phytochemical screening and of anthelmintic activity of *Pithecellobium dulce* leaves.

MATERIALS AND METHODS

Collection of the Plant Material

Pithecellobium dulce leaves were collected from the college ground, Mother Teresa

Pharmacy College, Sathupally, Khammam, Telangana.

Preparation of Pithecellobium dulce leaves powder

Plant leaves are collected and air-dried because to prevent them from direct sunlight impact to minimize undesirable chemical reactions of plant metabolites. Dry conditions are crucial to prevent the formation of artifacts as a result of microbial fermentation and subsequent degradation of the plant metabolites. Hence in the present study, leaves are dried in shade and then powder with a mechanical grinder. The powder was passing through sieve number 44 and stored in an airtight container for further studies.

Preliminary Phytochemical Screening Preparation of Plant Extract

The coarse powder of the plant material was weighed (20g) and placed into the earthy-colored glass bottles. The coarse powder was exposed to extraction in 250ml of every one of petroleum ether, chloroform, and methanol solvents independently. At that point, the solvents were added to it. At that point, the containers were fixed with aluminum foil and kept in a research center shaker at room temperature, and the flasks were shaken for 5 days. At last, the concentrate was sifted through numerous layers of muslin fabric for coarse filtration. The coarse filtrate was then separated through Whatman filter paper number 1. They got filtrate was vanished in a vacuum turning evaporator under decreased pressing factor at 40ºC until the filtrate was diminished to 33% of the beginning filtrate volume and the concentrated concentrates were additionally dissipated to get dry concentrates. A piece of dry concentrates was re-disintegrated in dimethyl sulfoxide (DMSO) and was put away in plug glass bottles and another part was kept as such in hermetically sealed containers at 4ºC for additional examination.

Phytochemical Screening

The phytochemical screening establishes the presence of different compounds possessing therapeutic effects. The different solvent extracts of *Pithecellobium dulce* leaves were used for screening the presence of carbohydrate, glycosides, alkaloids, flavonoids, steroids, tannins, saponins, phenol, protein, quinone, anthraquinone, sugar and terpenoids according to standard procedures[12].

Anthelmintic activity of *Pithecellobium dulce* leaves:

Preparation of earthworms:

Indian earthworm *Pheretima posthuma* was used to observe the anthelmintic Activity (Annelida) were collected from the waterlogged areas of soil at Sathupally. The earthworm *Pheretima posthuma* is one of the most crucial soil invertebrates that promoting soil fertility. Their feeding and burrowing activities help in the breakdown of organic matter and release nutrients and improve the aeration, drainage, and aggregation of soil. The average sizes of *Pheretima posthuma* were 7-9 cm with a weight of 0.8-2.25g were used for all experimental work. They were washed with normal saline to remove all the fecal matter surrounding their body. The earthworms resembled the intestinal roundworm parasites of human beings both anatomically and physiologically and hence were used to study the anthelmintic activity [13,14].

Experimental method:

These worms were separated into different groups containing five earthworms in each group. The plant extract was dissolved in a minimum quantity of 2% v/v Tween 80 and the volume was made up to 10 ml with normal saline for making the concentration of 10, 20and 50mg/ml. All the plant extract concentrations and the standard drug solution were freshly prepared before conducting the experiments. All the earthworms were washed with normal saline solution before they were released into 10ml respective formulation, vehicle (2% v/v Tween 80 in normal saline), and Piperazine Citrate (10, 20, and 50 mg/ml) and plant extract (10, 20 and 50 mg/ml) the anthelmintic activity was prepared. 10ml formulations containing three different concentrations of methanolic extract (10, 20, and 50 mg/ml) were prepared and taken in different Petri plates and five earthworms were placed in the solutions. Observations were noted for the time taken to paralysis and the death of individual worms. Paralysis was noted when the worms do not revive even in normal saline and death was concluded when the worms lost their motility followed by fading away of their body color. Time for the death of worms was observed after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50°C stimulated and induced movements if the worm was alive.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of plants was predominant to the detection of bioactive principles which is a new source of therapeutically and industrially valuable compounds that may lead to the discovery of new drugs. In the present study, the presence of phytochemicals were screened with the petroleum ether, chloroform, and methanol extracts of the *Pithecellobium dulce* leaves and the results are shown in Table1.Crude extracts and medicines are manufactured based on the principles of natural compound seven by pharmaceutical companies, which may lead to large-scale exposure of humans to natural products. Presence or absence of important bioactive compounds in an Extracts were identified by color reactions with specific chemicals, this procedure is simple for-preliminary pre-requisite before going to the phytochemical investigation. Hence, in the present work, the crude extracts obtained by petroleum ether, chloroform, and methanol solvents were screened for the presence of phytochemicals. The methanol extract shows the presence of steroids, saponins, flavonoids, phenols, proteins, glycosides, and terpenoids. Saponins have health benefits such as lower cholesterol, antimicrobial, anti-inflammatory, and anticancer properties[15].

Table no 1 Preliminary phytochemical screening of *Pithecellobium dulce* leave

Test	Petroleum ether	Chloroform	Methanol
Alkaloids	+	+	+
Steroids	-	-	+
Tannins	-	+	+
Saponins	+	+	+
Phenols	+	-	+
Flavonoids	+	+	+
Terpenoids	-	+	+
Glycosides	-	+	+
Proteins	-	-	-

+ indicates the presence of the phytochemical;

- indicates the absence of the phytochemical

Phenolic compounds have biological and pharmacological properties such as anti-

inflammatory, antioxidant, and antimutagenic, and anticarcinogenic activities. Flavonoids are secondary metabolite having various pharmacological properties such as anti-oxidative, anti-fungal, anti-inflammatory and diuretic actions[16-18].This research finding highlights that methanolic extracts of *Pithecellobium dulce* leave had the highest number of phytochemicals compared to other solvent extracts. Hence, methanolic extracts of *Pithecellobium dulce* leaves hold the great potential to treat various human diseases and has profound medical applicability.

The mechanism for anthelmintic activity of plant extracts is due to the presence of secondary metabolites bind to free proteins in the gastrointestinal tract of the host animal and glycoprotein on the cuticle of the parasite. The result of anthelmintic activity on earthworm *Pheretima posthuma* was shown in Table-2 reveals that different concentrations used have shown paralysis and death of worms and it was compared in the same concentration with Piperazine citrate. This standard drug may cause hyperpolarization of worms muscle by GABA agonistic action opening Chloride ion channels that cause relaxation and depresses responsiveness to contractile action of Acetylcholine. By increasing, chloride ion conductance of worm muscle membrane initiates hyperpolarization and reduced excitability that led to muscle relaxation and flaccid paralysis.

Table No 2. Anthelmintic activity of Methanolic extract of *Pithecellobium dulce* leaves

Groups	Dose in Concentration(mg/ml)	Time of paralysis(min)	Time of death(min)
Control	-	-	-
Methanolic extract	10	23.23±4.26	28.15 ± 2.25
	20	17.15± 3.58	22.06±2.76
	50	10.27 ± 2.16	13.38 ± 2.57
Standard drug	10	11.16±1.05	13.12 ± 2.59
	20	9±2.35	11.22 ± 1.34
	50	6 ±0.27	8.49 ± 0.28

The methanolic extracts of *Thuja occidentalis* leaves and standard drug solution not only illustrate paralysis but also causes the death of worms especially at a higher concentration of 50 mg/ml, in very less time was shown in Fig 1,2.

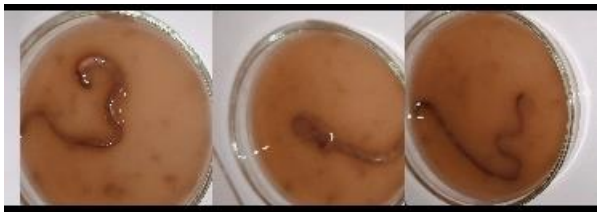

Figure no 1: Anthelmintic activity of the methanolic extract

Figure no 2: Anthelmintic activity of the standard drug

In addition, Tannins or their metabolites have an undeviating effect on the possibility of the pre-parasitic stages of helminths and other phytochemical constituents might be responsible for an anthelmintic activity include flavonoids and terpenoids. This present research work shows the presence of different phytochemical constituents like triterpenoids, steroids, glycosides, anthraquinone, flavonoids, and proteins with biological activity that can be a valuable therapeutic index. The plant extracts can be used for further isolation of compounds for their anthelmintic activity.

CONCLUSION

The presence of phytoconstituents, such as phenols and flavonoids in plants, indicates the possibility of antioxidant activity, and this activity will help in preventing a number of diseases through free radical scavenging activity. Since the plant *Pithecellobium dulce* leaves have been used in the treatment of different ailments, the medicinal roles of this plant could be related to identifying bioactive compounds. The present analyses suggest that *Pithecellobium dulce* leaves contain potentially health-protective phytochemical compounds with a potent source of natural antioxidants and antibacterial activities that may be clinically promising. The present results will form the basis for the collection of new plant species for further investigation in the potential discovery of new bioactive compounds. Further studies are needed for the in-vitro model are required to find out and to establish the effectiveness and pharmacological rationale for the use of plant leaves as an anthelmintic drug. The

biological parameter can be concluded that the plant *Pithecellobium dulce* leaves have significant anthelmintic activity.

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