

PHYTOCHEMICAL SCREENING AND IN-VITRO ANTHELMINTIC ACTIVITY OF *THUJA OCCIDENTALIS* LEAVES

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ABSTRACT

The present research work deals with determination of phytochemical constituents and anthelmintic activity of *Thuja occidentalis* leaves of Cupressaceae family were identified in order to relate their presence with bioactivities of the plants. The research find highlights that methanolic extracts of *Thuja occidentalis* had the highest number of phytochemicals compared to other solvent extracts. Hence, methanolic extracts of *Thuja occidentalis* holds the greatest potential to treat various human diseases and has 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 in to 10ml of 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 highest concentrations of 50mg/ml, a 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 methanolic extract of Indian medicinal plants exhibits potentiality and have maximum anthelmintic activity.

KEYWORDS: *Thuja occidentalis* leaves, Phytochemical screening, *Pheretima posthuma*, Piperazine citrate.

1. INTRODUCTION

The use of medicinal products and supplements has increased exceedingly over the past decades, not less than 80% of world population are depends on medicinal plant products for their primary health. In many developing countries, major proportion of the population are depends on traditional practitioners and using medicinal plant products in order to get their health in good conditions.^[1] *Thuja occidentalis*, known as white cedar, is native to eastern North America and is grown in Europe as an ornamental tree.^[2,3] Getting knowledge about the chemical constituents of plant is an essential for the usage in medicine and also for the synthesis of complex chemical components.^[4] An association between the phytoconstituents and their bioactivity of the plants are desirable to know for the synthesis of compounds with specific activities to treat various health issues and chronic diseases as well. *Thuja* leaves are utilized for the treatment of various diseases like respiratory tract infections such as bronchitis, bacterial skin infections, and cold sores.^[5] It is also utilized for the treatment osteoarthritis and a nerve disease that affects the face called trigeminal neuralgia, also used for the treatment of Cystitis, psoriasis, uterine

carcinomas, amenorrhea and rheumatism.^[6,7] A number of plant species that thrive in hostile environment replete with bacteria, fungi or virus synthesize defensive natural products against these pathogens, they may also exhibits bactericidal, fungicidal or virucidal activity in human beings.^[8-9]

Parasitic infections are common in the tropical regions that infect humans. Parasitic diseases disable their hosts and render them incapable of leading normal life and 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 research work was carried out on 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* were used for evaluation of anthelmintic activity in vitro. The present research work deals with evaluation of phytochemical screening of *Thuja occidentalis* leaves

and prove traditional anthelmintic medicinal properties of plants with a view to focus on future studies.

MATERIAL AND METHODS

Collection of the Plant Material

Thuja occidentalis leaves were collected from the college ground, Mother Teresa Pharmacy College, Sathupally, Khammam, Telangana.

Preparation of *Thuja occidentalis* leave powder

Plant leaves are collected and air dried because to prevent it 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 weighted (20g) and placed into the earthy colored glass bottles. The coarse powder was exposed to extraction in 250ml 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 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 40°C 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 were re-disintegrated in dimethyl sulfoxide (DMSO) and were put away in plug glass bottles and another part was kept as such in hermetically sealed containers at 4°C for additional examination.

Phytochemical Screening

The phytochemical screening establishes regarding the presence of different compounds possessing therapeutic effects. The different solvent extracts of *Thuja occidentalis* leaves were used for screening the presence of carbohydrate, glycosides, alkaloids, flavonoids, steroids, tannins, saponins, phenol, protein, quinone, anthroquinone, sugar and terpenoids according to standard procedures.^[12-16]

Anthelmintic activity of *Thuja occidentalis* leave

Preparation of earthworms

Indian earthworm *Pheretima posthuma*, were used to observe the anthelmintic Activity (Annelida) were collected from the water logged areas of soil at Sathupally. The earth worm *Pheretima posthuma* are one

of the most crucial soil invertebrate that promoting soil fertility. Their feeding and burrowing activities helps in breakdown of organic matter and release nutrients and improve aeration, drainage and aggregation of soil. The average sizes of *Pheretima posthuma* were 7-9 cm with weight 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 where used to study the anthelmintic activity.^[17,18]

Experimental method

These worms were separated into the different groups containing five earth worms in each group. The plant extract was dissolved in minimum quantity of 2% v/v Tween 80 and the volume was make upto 10 ml with normal saline for making the concentration of 10, 20 and 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 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 with fading away of their body color. Time for death of worms were 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 *Thuja occidentalis* leaves and the results are shown in Table 1. Crude extracts and medicines are manufactured based on the principles of natural compounds even by pharmaceutical companies, 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 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 alkaloids, steroids, saponins, flavonoids, phenols and terpenoids. Saponins have health benefits such as lower cholesterol, antimicrobial, anti-inflammatory and anticancer properties [19-21]. Many herbal medicinal researches have established saponins as the active components and their contributions to the health benefits of foods such as soybeans and garlic.

Table 1: Preliminary phytochemical screening of *Thuja occidentalis* leaves.

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. This research finding highlights that methanolic extracts of *Thuja occidentalis* leaves had the highest number of phytochemicals compared to other solvent extracts. Hence, methanolic extracts of *Thuja occidentalis* leaves holds the great potential to treat various human diseases and has profound medical applicability.

The mechanism for anthelmintic activity of plant extracts are due to presence of secondary metabolites bind to free proteins in the gastrointestinal tract of 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 2: Anthelmintic activity of Methanolic extract of *Thuja occidentalis* leaves.

Groups	Dose in Concentration (mg/ml)	Time of paralysis(min)	Time of death(min)
Control	-	-	-
	10	20.23±3.22	25.15 ± 2.25
Methanolic extract	20	16.15± 2.56	21.06±2.11
	50	9.22 ± 2.11	12.32 ± 2.55
	10	12.16 ± 1.05	15.12 ± 2.49
Standard drug	20	10±2.25	13.22 ± 1.34
	50	7 ± 0.40	9.19 ± 0.25

The methanolic extracts of *Thuja occidentalis* leaves and standard drug solution not only illustrate paralysis, but also causes death of worms especially at higher

concentration of 50 mg/ml, in very less time was shown in Fig 1,2.



Figure 1: Anthelmintic activity of methanolic extract.



Figure 2: Anthelmintic activity of standard drug.

In addition, Tannins or their metabolites have an undeviating effect on the possibility of the pre-parasitic stages of helminthes 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 alkaloids, steroids, saponins, flavonoids, phenols and terpenoids with biological activity that can be 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 *Thuja occidentalis* leaves has been used in the treatment of different ailments, the medicinal roles of this plant could be related to identify bioactive compounds. The present analyses suggest that *Thuja occidentalis* 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 collection of new plant species for further investigation in the potential discovery of new bioactive compounds. Further studies are need for in-vitro model are required to find out and to establish effectiveness and pharmacological rationale for the use of plant leaves as anthelmintic drug. Biological parameter can be concluded that the plant *Thuja occidentalis* leaves has significant anthelmintic activity

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