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PHYTOCHEMICAL SCREENING AND ANTHELMINTIC ACTIVITY OF LEAVES OF *CEDRUS DEODARA* (ROXB.)

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ABSTRACT

Helminthic infections are chronic illnesses both in human beings as well as in cattle. *Pheritima postuma* is a known helminthic which is commonly known as earth-worms. Parasite worms also infect livestock and crops, affecting food production with a resultant economic impact. Medicinal plants belong to the oldest known health care products that have been used by mankind all over the world in the form of folklore medicines or traditional medicines or ethno medicines. The use of herbal drugs extracts and their remedies have significantly increased throughout the world and the green revolution in terms of herbal medicines has now achieved astonishing popularity against the increasing use of synthetic medicines. **Objective:** To study phytochemical screening and evaluation of anthelmintic activity of various prepared extracts obtained from the leaves of *Cedrus Deodara* (Roxb.) against adult earth worms *Pheritima postuma*. **Material and Methods:** Three concentrations (10 mg/ ml, 25 mg/ ml and 50 mg/ ml) of various prepared extracts such as Petroleum ether extract, chloroform extract, ethyl acetate extract and methanol extract were tested in earth worms and results were expressed in terms of time for paralysis and time for death of worms. Albendazole (10 mg/ ml) was used as reference standard and tween 80 (0.5%) as a vehicle control group. **Result:** Among all the extracts tested at 10 mg/ ml, 25 mg/ ml, 50 mg/ ml concentrations, petroleum ether extract shows better anthelmintic activity when compared with other prepared extracts and with the standard drug albendazole. It shows marked significance effect. **Conclusion:** This study has demonstrated that the pet.ether leaves extract of *Cedrus deodara* possess significant *in vitro* anti-worm activity at the tested concentrations.

KEYWORDS: Albendazole, Anthelmintic, *Cedrus Deodara.*

INTRODUCTION

Helminthic infections are among the most common infections in man, affecting a large proportion of the world population. In developing countries they pose a large threat to public health and contribute to the prevalence of malnutrition, anaemia, eosinophillia and pneumonia.[1] An integrated approach is required for the effective control of helminthes which includes strategic and tactical use of anthelmintics which remains the corner stone to this end and careful management of grazing lands including control of stocking rates and appropriate rotation strategies. Amongst various anthelmintic plants *Cedrus deodara* is the most potent plant for curing helminthic infections. *Cedrus deodara* is an evergreen conifer tree reaching upto 85 m in height with almost rough black furrowed bark and spreading branches, shoots are dimorphic, but some trees or branches are habitually bear flowers of one sex.[2]

All parts of *Cedrus deodara* are bitter, hot, slightly pungent, oleaginous, useful in inflammations, dyspepsia, insomnia, cough[3], fever, urinary discharges, ozoena, bronchitis, itching, elephantiasis, tuberculous glands, leucoderma, opthalmia, piles, disorders of the mind, diseases of the skin[4] and of the blood.[5-6] The leaves lessen the inflammation applied in tuberculosis glands. The wood is bitter, carminative, expectorant and useful in rheumatism, piles, epilepsy, stones in the kidney and bladder, useful in fever, piles pulmonary complaints, and prolapsus recti.[7-8] The oil is antiseptic and used in skin diseases, sores, wounds and ulcers[9] and also for headache, fever, urogenital diseases, piles and as a carminative, antidiarrhoeal, diaphoretic, diuretic and insecticide. The heartwood is used for similar purposes and as in anti-inflammatory activity[10], Immunomodulatory activity[11-12], Anti-malarial activity[13], Mast cell stabilizing and lipoxygenase inhibitory activity[14], Anti-allergic activity[15],Anti-tubercular activity[16], Anti-diabetic Activity[17], Anti-oxidant activity[18-19], laxative[20], diuretic, sedative, Anti-bacterial activity[21], Anti-fungal[22], cardio tonic[23] and for many other disorders. The leaves are bitter and acrid and used mainly in inflammation[24-25] and Anti-hypertension.[26-27]

MATERIAL AND METHODS

Collection of plant material

The plant material *Cedrus deodara* investigated in the present study was collected from Solan Himachal pardesh in the months of March- April, 2013. The plant was identified and authenticated by Dr. Yashwant Singh Parmar University of Horticulture & forestry (Nauni),

near Solan, Himachal Pardesh (H.P), vide Book no. 2916, Reciept no. 046 and the said sample is linked to UHF- Herbarium with field book No. 13426 and 13427 respectively. The herbarium is kept at Nauni University for further reference.

Collection of Worms

The Indian earthworm *Pheritima postuma* was collected from Agriculture office, Chambaghat, Solan, (H.P).

Earth worms[28-31]

The Indian adult earthworms *Pheritima postuma* collected from moist soil and washed with normal saline to remove all adhering faecal matter which were further used for the anthelminthic study. The earthworm of 3-5 cm in length and 0.1- 0.2 cm in width were used for all experimental protocol due to its anatomical and physiological resemblance with intestinal roundworm parasite present in body of human beings.

Chemicals and Reagents

The following drugs and chemicals were used.

Drug: Albendazole

Chemicals: Methanol, ammonia solution, Dragendorff's reagent, Mayer's reagent, Molisch's reagent, sulphuric acid, acetic anhydride, Ethanol, Million's reagent, Tween 80, hydrochloric acid, Petroleum Ether, Ethyl acetate, Chloroform.

Preparation of plant extracts

The collected plant material was washed thoroughly in water, dried under shade and ground to coarse powder in electric grinder. Powdered material was then extracted successively in Soxhlet apparatus, using Petroleum Ether, Chloroform, Ethyl acetate, methanol respectively. The extracts were further concentrated to semisolid mass and stored in airtight container in a refrigerator till further use.

Phytochemical screening of all the prepared extracts[32]

The preliminary phytochemical screening of the leaves of *Cedrus deodara* plant extract mainly done for the evaluation of the various phytoconstituents such as steroids, alkaloids, glycosides, saponins were present in plant extracts prepared in various solvents such as petroleum ether, chloroform, methanol and ethyl acetate of *Cedrus deodara* shown in **Table no.1**.

Test for alkaloids

The small portion of prepared plant extracts were stored separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal reagents, such as Mayer's reagent (cream precipitate) and Dragendorff's reagent (orange brown precipitate).

Test for carbohydrates and glycosides

Small quantities of plant extract were dissolved separately in 5 ml of distilled water and filtered. The filtrate may be subjected to Molisch's test to detect the presence of carbohydrates. Another small portion of extract was hydrolyzed with dilute hydrochloric acid for few hours in a water-bath and was subjected to Liebermann- Burchard's, legal and Borntrager's test to detect the presence of different glycosides.

Test for flavonoids

5 ml of dilute ammonia solution were added to a portion of aqueous filtrate of plant extract followed by addition of concentrated H_2SO_4. A yellow coloration is observed in extract which indicates the presence of flavonoids. The yellow coloration is further disappeared on standing.

Test for steroids

2 ml of acetic anhydride was added to 0.5 g plant extract with 2 ml H_2SO_4. The colour changed from violet to blue or green in samples which indicates the presence of steroids.

Test for terpenoids (salkowski test)

5 ml of extract was mixed with 2 ml of chloroform and then concentrated H_2SO_4 (3 ml) was carefully added from the side of the test tube to form a layer. A reddish brown coloration was observed at the interface of two liquid layers which indicates the presence of terpenoids.

Test for saponins

About 1 ml of plant extract was diluted with 20 ml of distilled water and shaken in graduated flask for 15 minutes. One cm layer of foam indicates the presence of saponins.

Test for proteins Million's reaction: Million's reagent (mercuric nitrate in nitric acid containing the traces of nitrous acid) usually yields a white precipitate on addition to a protein solution which turns red on heating. This reaction is characteristic of phenols (e.g. the phenolic amino acid tyrosine).

Anthelmintic activity

1. Standard solution

Albendazole (10 mg/ ml, 25 mg/ ml and 50mg/ ml) was administered as standard solution.

2. Test solution

The different concentrations (10, 25 and 50 mg/ ml) of petroleum ether (PE), chloroform extracts (C), ethyl acetate (EA), methanol extracts (M) of leaves of *Cedrus deodara* were prepared. All the extracts and the standard drug albendazole solution were freshly prepared before starting the experiments.

3. Experimental design[33]

All the extracts were suspended in 0.5% solution of tween 80 which is prepared in distilled water. All the solutions and extracts were freshly prepared before the commencement of the experiment. Sixteen groups each containing six earthworms were released into 10 ml of desired formulation as; Group 1 were the control worms placed in vehicle 0.5% Tween 80 in distilled water; Groups 2-4 received petroleum ether (PE) extracts of *Cedrus deodara* at 10, 25 and 50 mg/ ml concentrations respectively; Group 5-7 were treated with chloroform extracts (C) at 10, 25 and 50 mg/ ml concentrations respectively; Group 8-10 were treated with ethyl acetate extract (EA) at 10, 25 and 50 mg/ ml concentrations respectively; Group 11-13 were treated with methanol extracts (M) at 10, 25 and 50 mg/ ml concentrations respectively and Group 14-16 serves as standard and was treated with Albendazole (10, 25 and 50 mg/ ml). Final volume was adjusted to 10 ml in each petridish. Observations were made on the basis of the time taken to paralysis and cause death of individual worms during the test period. Paralysis was said to occur when the worms did not survive even in normal saline. Death was concluded when the worms lost their motility followed by fading away of their body colour.

RESULTS AND DISCUSSIONS

Table No. 1: Preliminary Phytochemical screening of *Cedrus deodara* leaves extracts.

S.No.	Phytochemical tests	Petroleum ether Extract	Chloroform extract	Ethyl acetate extract	Methanol extract
1.	Carbohydrates	-	-	-	+
2	Fats & Oils	-	-	+	+
3.	Proteins	-	+	-	+
4.	Amino acids	-	-	-	-
5.	Alkaloids	++	++	+	++
6.	Glycosides	-	-	+	+

7.	Flavonoids	++	-	++	+
8.	Saponins	++	+	+	+
9.	Tannins and Phenolic compounds	++	+	+	+
10.	Triterpenoids	+	+	+	+
11.	Steroids	+	-	+	-

Note: (+) means positive, (-) means negative.

Preliminary phytochemical screening of all the prepared extracts showed the presence of alkaloids, tannins, saponins, flavonoids and steroids. These phytoconstituents are responsible for the anthelmintic activity. These results supports the traditional use of the plant.

In –vitro Anthelmintic Activity

Effect of different extracts at time of paralysis

Petroleum ether, Chloroform, Ethyl acetate and Methanol extracts showed better anthelmintic activity when compared with standard drug at the same concentrations. Petroleum ether extract took the least time to cause paralysis of the worms followed by Methanol, Ethyl acetate and chloroform respectively when compared with Albendazole at 10, 25 and 50 mg/ ml dose. It is clearly shown in **Table No.2.**

Effect of different extracts at time of death

Petroleum ether, Chloroform, Ethyl acetate and Methanol extracts showed better anthelmintic activity when compared with standard synthetic drug at the same concentration. Pet.ether extract took the least time to cause death of the worms followed by Methanol, Ethyl acetate and chloroform respectively. If 10 mg/ ml dose of albendazole is compared with Pet. Ether, chloroform, methanol and ethyl acetate extract then it can be concluded that plant extract contains better potency as compared to synthetic drug for anthelmintic activity. It is clearly shown in **Table No.3.**

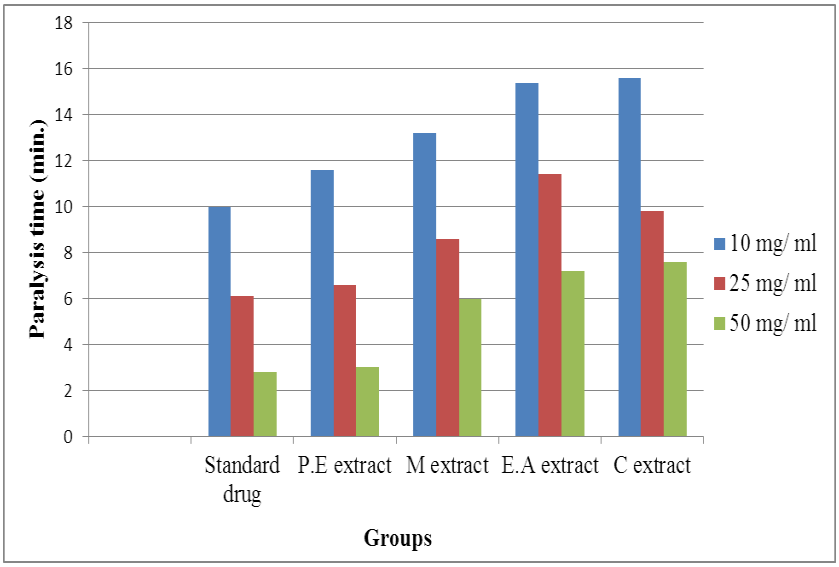
Table No. 2: Anthelmintic activity of different extracts of *Cedrus deodara* leaves at time of paralysis.

Treatment	Concentration (mg/ ml)	Paralysis time (min.)
Vehicle (Tween 80)	---	---
Albendazole	10	10±3.807
	25	6.1±2.808
	50	2.8±1.032
Pet. ether extract	10	11.6±2.863
	25	6.6±1.516
	50	3±1.0
Methanol extract	10	13.2±4.722

	25	8.6±4.219
	50	6±2.236
Ethyl acetate extract	10	15.4±2.607
	25	11.4±1.516
	50	7.2±2.683
Chloroform extract	10	15.6±7.127
	25	9.8±4.816
	50	7.6±6.107

All values represents in mean ± SD; n=6 in each group.

Vehicle worms were alive to 24 hrs under observation.



Graph-1. Time taken for paralysis after treatment with *Cedrus deodara* leaf extracts where P.E –Petroleum ether Extract, M-Methanol Extract, EA- Ethyl acetate Extract, C- Chloroform extract, Standard drug- Albendazole. The number represents time in minutes.

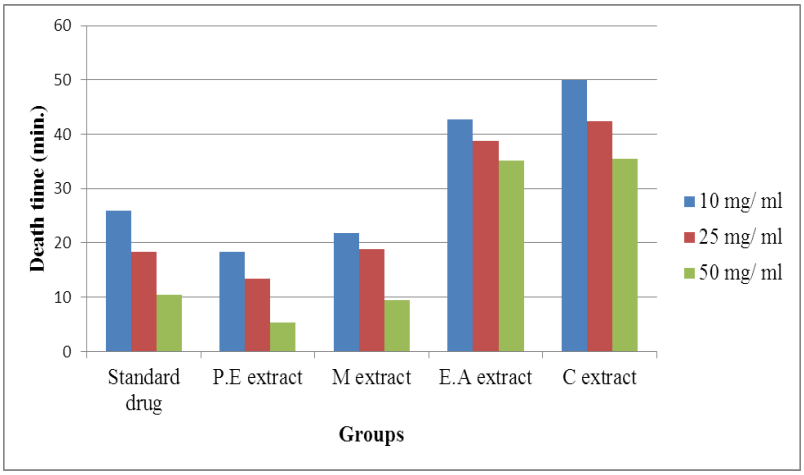
Table No. 3: Anthelmintic activity of different extracts of *Cedrus deodara* leaves at the time of death of earthworms.

Treatment	Concentration (mg/ ml)	Death time (min)
Vehicle (Tween 80)	---	---
Albendazole	10	26±6.964
	25	18.4±3.682
	50	10.4±3.507
Pet. ether extract	10	18.4±3.209
	25	13.4±3.286
	50	5.4±1.140
Methanol extract	10	21.8±2.774
	25	18.8±2.167
	50	9.4±3.507
Ethyl acetate extract	10	42.8±10.281

	25	38.8±9.731
	50	35.2±9.284
Chloroform extract	10	50±3.807
	25	42.4±7.300
	50	35.4±9.208

All values represents in mean ± SD; n=6 in each group.

Vehicle worms were alive to 24 hrs under observation.



Graph-2. Time taken for death after treatment with *Cedrus deodara* leaf extracts where P.E –Petroleum ether Extract, M-Methanol Extract, EA- Ethyl acetate Extract, C-Chloroform extract, Standard drug- Albendazole. The number represents time in minutes.

As displayed in Graph -1 and Graph -2 petroleum ether extract and methanol extract of leaves exhibited significant anthelmintic activity in dose dependent when compared with reference standard Albendazole. In comparison with methanol extract petroleum ether extract of leaves in concentration of 50 mg/ ml was found to be paralysis and death of worm in 3±1.0 and 5.4±1.140 minutes time respectively which is potentially much effective as compare with standard reference drug Albendazole. The compound constituents responsible for anthelmintic activity were not investigated however preliminary phytochemical screening of extracts give positive test for steroids, alkaloids, terpenoides, saponins, tannins, flavonoids. The role of flavonoids, saponins, alkaloids and steroids are responsible for anthelmintic activity. The comparison between treated groups with standards was carried out using one way ANOVA test. All the results were found to be significant with P value less than 0.0001 ($P< 0.0001$).

DISCUSSION

The data revealed that the various extracts showed paralysis and time of death at a concentration of 50 mg/ ml, 25 mg/ ml, 10 mg/ ml in concentration dependent manner. The

test concentration of all the extracts showed marked degree of anthelmintic activity with maximum activity of petroleum ether extract. The anthelmintic effect of extracts is comparable to the effect produced by the standard drug albendazole.

Tremendous advances have been made during the previous decade and substantial numbers of synthetic precursors have been derived to cope up the damage caused by parasite. But unfortunately no effective medicine has been developed so far. Some serious side effects of drug and development of resistance drives the severity of infection to the next level. These factors paved the way for herbal remedies as alternative anthelminthics. The result of this study has shown promising anthelmintic activity suggesting the possible use of *Cedrus deodara* extract in intestinal nematode control.

CONCLUSION

This study has demonstrated that the various leaf extract of *Cedrus deodara* possess significant *in vitro* anti-worm activity at the tested concentrations. The pet. ether extract shows maximum activity at all the tested concentration. Thus, the wormicidal activities of the extract against earthworm suggest that it could be effective against parasitic helminths of humans and animals. The experimental evidence obtained in this laboratory model could provide a rationale for the traditional use of the plant as anthelminth. However, further studies are needed to isolate, characterize and evaluate the actual bioactive components and their mechanism of action. Also, studies on the toxicity, evaluation of the effect *in-vivo* and the establishment of adequate doses for human and animals are recommended.

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