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ANTIHELMINTHIC ACTIVITY OF DIFFERENT EXTRACTS OF *DENDROBIUM OCHREATUM*

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ABSTRACT

Dendrobium ochreatum whole plant was used in this study, each of plant parts like root stem and leaf powder was subjected to extraction using three different solvents i.e. hexane, chloroform and ethanol successively in gradient elution technique. All total nine extracts were obtained which were further proceeded to antihelminthics activity against Indian earthworm comparing with standard drug Mebendazole. Extracts were diluted and different concentrations of plant extracts were prepared in distilled water dissolved in 6% Dimethyl Sulfoxide (DMSO). The concentrations prepared were 1000 µg/ml, 800 µg/ml, 600 µg/ml and 400 µg/ml for all the extracts. Observations were made for the time taken to cause paralysis and death of individual worms. The paralyzing and death times were noted and their averages were calculated for each plate containing 6 worms for an individual concentration followed by calculating mean paralyzing and death. The results signify that out of nine extracts the ethanolic extracts of root exhibited comparatively better activity among all extracts in terms of paralyzing (31±0.45 min) and death time (58±0.63 min).

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Introduction

Helmenthiasis is generally prevalent in third world countries due to poor hygiene. Antihelminthics either kill or expel intestinal parasitic worms. Their mechanism includes interfering with parasite's carbohydrate metabolism, inhibiting respiratory enzyme, and blocking neuromuscular action. [1] symptoms like retarded cognitive development, iron-deficiency anemia, and abdominal pains are related to helminths infections.[2]

Antihelminthics drugs have wide variety of mechanisms. Some drugs act on neuromuscular transmission of these worms. Drugs like Levamisole, pyrantel pamoate acts on acetylcholine receptor and cause spastic paralysis. Piperazine is a GABA (gamma-amino-butyric acid) agonist which acts on receptor of nematode muscles and causes flaccid paralysis. Opening of glutamate-gated chloride (GluCl) channels is initiated by avermectins which produce paralysis of pharyngeal pumping. Praziquantel increases permeability of calcium. Binding to beta-tubulin of worms' cells is initiated by benzimidazole derivatives and ultimately inhibit the process of microtubule formation. Diethylcarbamazine blocks host and parasite, and involves in the process of sensitizing the microfilarae to phagocytosis. [3-8]

Due to high cost along with some complications related to synthetic drugs over the years, herbal medicines are choice of treatment for this infection. Various extracts of medicinal plants like *Swietenia mahagoni*, *Acalypha indica* L., *Euphorbia hirta*, *Piliostigma thonningii*, *Butea monosperma*, *Cucurbita maxima*, *P. granatum*, *Capparis decidua*, *Capparis spinosa*, *Anacardium occidentale*, *Mimusops elengi* Bark, *Cleome icosandra*, *Zingiber officinale*, *Piper longum*, *Trachyspermum ammi*, *Acorus calamus*, *Glycyrrhiza glabra*, *Cuminum cyminum*, and *Saussurea lappa* are now extensively used for treatment of helminthic infections. [9-14]

In this work, a rare species of orchid, *Dendrobium Ochreatum*, was used. Different parts of the plant (root, stem leaf) were extracted with different polarity solvents and all of nine extracts were subjected to *in vitro* antihelminthic study comparing with standard mebendazole.

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Materials and Methodology

Extraction

About 250 g of each of leaf, stem, and root powder was filled in the thimble and extracted successively with hexane, chloroform, ethanol using a Soxhlet extractor for 72 hr. The solution of the extract was filtered through Whatman filter paper no.1 and concentrated using rotary flash evaporator and stored in the refrigerator.

In vitro Antihelminthic activity

The earthworms were collected from Dharan -16, (NEPAL) near the college campus, then washed with normal saline to remove all earthy materials. The collected earthworms were kept in laboratory for further study. The activity was evaluated on earthworm *Phaeritima posthuma*. The different concentrations of plant extracts were prepared in distilled water dissolved in 6% Dimethyl Sulfoxide (DMSO). The concentrations prepared were 1000 µg/ml, 800 µg/ml, 600 µg/ml and 400 µg/ml for all the extracts. The standard drug (Mebendazole) was prepared in 6% DMSO at a dose level same as the extract's concentration. Those earthworms which served as normal control received 6% DMSO in water. 6 earthworm of 8-10 cm were placed in each petri dish containing 25 ml of test solutions of extracts received different concentration of 400 µg/ml, 600 µg/ml, 800 µg/ml, 1000 µg/ml plant aqueous extracts and same procedure applied for dilution of standard drug also. Observations were made for the time taken to cause paralysis and death of individual worms. The paralyzing and death times were noted and their averages were calculated for each plate containing 6 worms for an individual concentration. Death was concluded when the worms lost their motility followed by fading away of their body colors.

Results

Table 1. Assigned code of different extracts.

Sr. No.	Parts of *Dendrobium ochreatum*	Solvent used	Assigned code
1	Leaf	Hexane	LHDO
		Chloroform	LCDO
		Ethanol	LEDO
2	Stem	Hexane	SHDO
		Chloroform	SCDO
		Ethanol	SEDO
3	Root	Hexane	RHDO
		Chloroform	RCDO
		Ethanol	REDO

Table 2. *In vitro* Antihelminthic activity of different extracts of *Dendrobium ochreatum*.

Sr. No	Plant Extracts	Antihelminthic activity (*in vitro*)			
		Concentration (µg /ml)	Paralyzing time (min)	Death time (min)	Blank
1	LHDO	1000 µg/ml	55±0.39	74±0.23	–
		800 µg/ml	64± 0.46	82±0.75	
		600 µg/ml	70± 0.72	89±0.87	
		400 µg/ml	95± 0.87	105±1.08	
2	LCDO	1000 µg/ml	30±0.14	63±0.32	
		800 µg/ml	33± 0.78	75±0.65	
		600 µg/ml	35± 0.97	79±0.77	
		400 µg/ml	45± 1.04	93±0.98	
3	LEDO	1000 µg/ml	35± 0.23	78±0.12	
		800 µg/ml	45± 0.56	83±0.35	
		600 µg/ml	52± 0.78	98±0.67	
		400 µg/ml	63± 1.09	109±0.78	
4	SHDO	1000 µg/ml	36± 0.24	74±0.17	
		800 µg/ml	42± 0.45	81±0.36	
		600 µg/ml	57± 0.67	89±0.65	
		400 µg/ml	68± 0.78	102±0.78	

5	SCDO	1000 µg/ml	27± 0.45	64±0.15	
		800 µg/ml	45± 0.67	87±0.57	
		600 µg/ml	61± 0.78	92±0.64	
		400 µg/ml	77± 0.98	116±0.88	
6	SEDO	1000 µg/ml	32± 0.45	63±0.78	
		800 µg/ml	40±0.67	77±0.67	
		600 µg/ml	48±0.78	84±0.97	
		400 µg/ml	57±9.98	93±1.10	
7	RHDO	1000 µg/ml	31±0.34	60±0.37	
		800 µg/ml	38±0.56	72±0.45	
		600 µg/ml	42±0.67	79±0.65	
		400 µg/ml	50±0.94	91±0.87	
8	RCDO	1000 µg/ml	35±0.55	64±0.34	
		800 µg/ml	37±0.67	75±0.63	
		600 µg/ml	43±0.98	84±0.76	
		400 µg/ml	54±1.07	97±0.98	
9	REDO	1000 µg/ml	31±0.45	58±0.63	–
		800 µg/ml	37±0.56	72±0.77	
		600 µg/ml	43±0.69	86±0.89	
		400 µg/ml	52±0.97	93±1.07	
10	Mebendazole (standard)	1000ug/ml	18± 0.33	38±0.33	
		8000ug/ml	26±0.56	48±0.56	
		6000ug/ml	34±0.76	54±0.73	
		4000ug/ml	40±0.87	68±0.87	

Conclusion

All nine extracts were subjected for *in vitro* anthelmintic activity against Indian earthworm taking mebendazole as standard drug. The extracts were diluted in 400, 600 800, and 1000 µg/ml. Average paralyzing time and average death time was noted. Death was concluded when the worms lost their motility followed by fading away of their body colors. Out of nine extracts examined all the extracts more or less exhibited anthelmintic property but comparatively REDO has shown to some extent better activity as it required less paralyzing and death time in comparison to other extracts to paralyze and kill the worms **(Table 2)**. The anthelmintic activity suggests that all the extracts possess comparatively more or less same potency in terms of paralyzing and killing the worms in different dilutions, therefore out of nine extracts ethanolic extracts of root was found to be more active among all other extracts.

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