December 2007 Volume 3 Issue 3

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 3(3), 2007 [200-202]

Comparative anthelmintic activity of leaves and stems of *Clerodendron multiflorum* (Verbenaceae) Linn.

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Received: 30th November, 2007 ; Accepted: 5th December, 2007

ABSTRACT

All the extracts from the leaves and stems of *Clerodendron multiflorum* (Verbenaceae) were investigated for their anthelmintic activity against *Pheritima posthuma*. Each extract was studied for their dose dependant activity (20mg/ml, 40mg/ml, 60 mg/ml), which involved determination of the time of paralysis and time of death of the worms. The potency of extracts for the anthelmintic activity of stem and leaves of *Clerodendron multiflorum* was found to be methanolic extract>pet ether extract >chloroform extract>acetone extract. The methanol extract of both the parts i.e. stem and leaves were found to be more active. The stems of *Clerodendron multiflorum* showed more potent activity as compare to leaves. Albendazole (20mg/ml) and distilled water were included in the assay as standard drug and control, respectively.

KEYWORDS

Clerodendron multiflorum;
Pheritima posthuma;
In-vitro anthelmintic activity;
Venabenaceae.

INTRODUCTION

Clerodendron multiflorum(Verbenaceae) is a large bush or small tree reaching 9m high. Leaves are ovate, coarsely dentate. Flowers are white to pink in colour, moderate sized, fragrant and arranged so as to form a rounded terminal panicle[1,2]. The certain flavonoids were isolated from dried roots and flower as, rhamnopyrosyl (1-2)-2-D-glucopyranosyl-7-O-naringin-4-O-α and D-glucopyranoside-5-methyl ether 2,4-trihydroxy, 6-methoxy chalcone-4, 4-α-D Diglucoside, pecto linarigenin, hispidulin, apigenin[4]. From leaves steroid (24s) ethylcholesta-5, 22, 35-trine- 3β-ol, flavonoids as scutellarein and pectolinarigenin(4', 6-dimethyl scutellarein) were isolated. Ceryl alcohol, clerodendrin, clerosterol and clerodendrin were isolated from roots[3,4,6].

The leaves are given in stomach pain, diarrhoea, dyspepsia and neglected syphilitic[7]. Ethanolic extract of leaves exhibits mild hepatoprotective activity and *invitro* anthelmintic activity against tapeworms and round worms[6]. It also exhibited the antidiabetic, anti-fungal activity[9]. The plant also exhibited anticonvulsant activity[8,9] and prevented pregnancy an albino rats[11]. The roots are used as bitter tonic[2].

The anthelmintic activity of the plant was not yet evaluated experimentally. So present study reports anthelmintic activity various extracts of *Clerodendron multiflorum* leaves and stems.

MATERIALS AND METHOD

Plant material

The stems and leaves of *Clerodendron multiflorum*

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TABLE 1 : Anthelmintic activity of various extracts of stems of *Clerodendron multiflorum*

Treatment	Dose mg/ml	Time of paralysis(min) ± SEM	Time of Death (min)±SEM
PES	20	0.42±0.00577	2.33±0.0115
	40	0.37±0.00881	1.456±0.0145
	60	0.32±0.00577	1.25±0.01528
CHS	20	0.48±0.00577	4.03±0.0115
	40	0.346±0.01202	3.61±0.01129
	60	0.296± 0.0133	3.2±0.00577
ACS	20	0.656±0.01202	6.196±0.0318
	40	0.583±0.00378	5.363±0.0338
	60	0.483±0.00881	4.38±0.03283
MES	20	0.313±0.00881	1.52±0.00881
	40	0.284±0.000881	1.19±0.03844
	60	0.254±0.00202	1.01±0.00881
ALBENDAZOLE	20	0.083±0.000577	2.23±0.1453
	40	0.071±0.00115	1.24±0.00881
	60	0.065±0.00057	1.05±0.00577
CONTROL	5% DMF in normal saline		

PES-pet ether extract of stem, CHS-chloroform extract of stem, ACS- acetone extract of stem, MES-methanol extract of stem. SEM- standard error of mean.

(Verbenaceae) were collected from Ahmednagar district, Maharashtra (India) in August 2007. The plant specimen was authenticated from Botanical Survey of India, Pune (Voucher specimen no. CKS1).

Preparation of extracts

Dried and coarsely powdered stem and leaf parts (500g each) of *Clerodendron multiflorum* were separately subjected to successive extraction using petroleum ether, chloroform, acetone and methanol in Soxhlet extractor. The extracts of various parts were concentrated by vacuum distillation and then dried in open air.

Animals

Indian adult earthworms (*Pheretima posthuma*) collected from moist soil of the Government Horticulture Department, Kopargaon and washed with normal saline to remove all the feacal matter, were used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1- 0.2cm in width were used for all the experimental protocol due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being.

Drugs and chemicals

Albendazole(Pfizer Ltd., Bangalore), Petroleum ether(PCL, Pune), Chloroform (PCL, Pune), Acetone (PCL, Pune), Methanol A.R.(PCL, Pune), DMF(PCL,

TABLE 2 : Anthelmintic activity of various extracts of leaves of *Clerodendron multiflorum*

Treatment	Dose mg/ml	Time of paralysis (min) ±SEM	Time of death (min) ±SEM
PEL	20	0.28±0.01155	2.33±0.01155
	40	0.243±0.00881	1.39±0.1296
	60	0.220±0.00606	1.253±0.0088
CHL	20	0.363±0.0088	3.08±0.00881
	40	0.313±0.001528	2.51±0.01453
	60	0.305±0.001732	2.36±0.02186
ACL	20	0.566±0.01856	6.02±0.0176
	40	0.533±0.00881	5.45±0.0115
	60	0.502±0.00371	5.106±0.0088
MEL	20	0.233±0.00881	1.48±0.01202
	40	0.197±0.001764	0.96±0.00881
	60	0.0184±0.00152	0.87±0.00577
ALBENDAZOLE	20	0.081±0.00115	2.076±0.0393
	40	0.0701±0.00120	1.23±0.0066
	60	0.066±0.0019	1.033±0.0088
CONTROL	5% DMF in normal saline		

PEL- pet ether extract of Leaves, CHL- chloroform extract of Leaves, ACL- acetone extract of Leaves, MEL- methanol extract of Leaves. SEM- standard error of mean.

Pune), Saline water(Nurilife, Ahmedabad).

Anthelmintic activity[12,13,14,15]

In each case, six earthworms were released into 10ml of desired formulations as follows; vehicles(5% DMF in normal saline), Albendazole(20mg/ml), or total pet ether, chloroform, acetone and methanol extracts of leaf and stem *Clerodendron multiflorum*(20mg/ml, 40mg/ml, 60mg/ml) in normal saline containing 5%

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DMF. Observations were made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when the worms lost their motility followed with fading of their body colors.

RESULTS AND DISCUSSION

It is evident from the result shown in TABLE 1 and TABLE 2, all the extracts showed the dose dependant anthelmintic activity. The stems of *Clerodendron multiflorum* showed the significant anthelmintic activity as compared to the leaves. The potency of extracts for the anthelmintic activity of stem and leaves of *Clerodendron multiflorum* was found to be methanolic extract>pet ether extract>chloroform extract>acetone extract[14]. All the extracts of stems and leaves showed significant activity at a concentration of 20mg/ml. Results were comparable with the standard drug, Albendazole, at the same concentration. The function of the anthelmintic drugs like Albendazole is to cause paralysis of worms so that they are expelled in the feaces of human being and animals. The extracts not only demonstrated this property, they also caused death of the worms. The preliminary phytochemical investigation showed the presence of flavonoids, tannins in methanolic extract and tannins are responsible for the anthelmintic activity[16,17]. pH of the formulation (extract which is diluted to 10ml with normal saline containing 5% of DMF) was also found out during the activity which was found to be 5-6. And further study was carried out for isolation of the chemical constituent responsible for anthelmintic activity. In conclusion, these plants have been confirmed to display anthelmintic activity.

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