

***In-vitro* anthelmintic activity of stem bark and seed capsules of *Callistemon lanceolatus* (Sm.) Sweet**

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ABSTRACT

Aqueous and methanol extract of stem bark and seed capsules of *Callistemon lanceolatus* (Sm.) Sweet were evaluated for anthelmintic activity against Indian adult earthworm *Pheretima posthuma*. The paralysis and death time of earthworms was observed. Albendazole (20mg/ml) was used as standard and normal saline as control. The aqueous and methanol extracts of stem bark and seed capsules at the concentrations of 12.5, 25, 50, and 100 mg/ml showed anthelmintic activity in a dose dependant manner. Best activity was exhibited by aqueous extracts of seed capsules followed by methanol extracts of seed capsules at same concentrations and least activity was observed by aqueous extracts of stem bark when compared with standard. Preliminary phytochemical screening of both parts support the presence of phytoconstituents responsible for anthelmintic action.

Keywords: Helminthiasis, Stem bark, Seed capsules, Paralysis time, Death time,

INTRODUCTION

Helminthiasis or worm infection are among the most common infections in humans, affecting a large population of the World [1,2]. It is unevenly distributed disease in low income countries which affect large population and possess highest risk of morbidity because it is the major source of environmental contamination and transmission [3]. Anthelmintic drugs are used to expel or kill the infesting agent [4]. Albendazole is benzimidazole derivative that has been widely used in the treatment of worm infestations in both humans and animals [5]. The gastro-intestinal helminthes becomes resistant to currently available synthetic drugs therefore there is a foremost problem in treatment of helminthes diseases and causes an increasing demand towards natural anthelmintics [6]. *Callistemon lanceolatus* (Sm.) Sweet with synonym-*Callistemon citrinus* Curtis is a handsome shrub or small tree up to 7.5 m in height belonging to family Myrtaceae, indigenous to Queensland and New south wales, now frequently cultivated throughout India in gardens [7]. Leaves are lanceolate shaped sometime broadly up to 7.5 cm, long with prominent veins, midrib and oil glands, Flowers are crimson with dark red anthers [8]. Flowers are arranged in long spikes resembling like a traditional bottlebrush because of which it commonly known as 'Crimson Bottlebrush tree' [9,10]. Fresh stem bark is brown in colour with rough outer surface and reddish brown coloured smooth inner surface. On drying bark attain curved curvature. Fresh Seed capsules are hard, woody, greenish coloured and mature are brown coloured. Traditionally plant is used in treatment of genitourinary and kidney infections, bleeding ulcer, gout, arthritis, as insecticidal, pesticides and as bee repellent [7]. The plant has been proved to exhibit anticholinesterase activity [11], hepatoprotective [12], inhibit elastase activity [13], Cardioprotective [14], Antidiabetic [15], hypolipidemic [16], antioxidant [17], antispasmodic [18,19]. The present study investigates comparison of anthelmintic activity of the stem bark and seed capsules to justify the use of the plant in the treatment of helminthiasis.

MATERIALS AND METHODS

Collection and Identification of plant

Plant of *Callistemon lanceolatus* (Sm.) Sweet were collected from campus of Guru Jambheshwar University of Science & Technology, Hisar in the month of August, 2012. A voucher specimen has been retained in Department of Pharmaceutical Science, Guru Jambheshwar University of Science & Technology, Hisar and was identified by Dr. HB Singh, Scientist and Head, Raw material Herbarium & Museum NISCAIR, New Delhi, India with wide ref. No. NISCAIR/RHMD/Consult/2012-13/2072/80. Stem bark and seed capsules were used to carry out experimental work.

Preparation of extracts

Shade dried coarse powder of stem bark seed capsules, free of any foreign matter were taken. Prepared methanolic extract by successive solvent extraction in methanol for 6 hours. Removed the marc and prepared the aqueous extract by dipping the dried powder in distilled water for 6 days. Stored the extract in dessicator for further use.

Phytochemical screening

The Phytochemical screening of methanolic and aqueous extracts of stem bark and seed capsules of *Callistemon lanceolatus* (Sm.) Sweet was performed to detect the presence of various phytoconstituents in the plant according to standard procedure [20,21,22].

Collection of test animals

Adult Indian earthworm (*Pheretima posthuma*) was used for study, because of its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. Easy availability of earthworms prompts their extensive use for preliminary in vitro evaluation of anthelmintic compounds. Adult Indian earthworms were collected from Agronomy Department of Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Hisar (Haryana) and identified by Dr. Thakral (Senior Scientist), Agronomy Department, CCSHAU, Hisar. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for whole experimental protocol.

Sample preparation

All the test solutions and standard drug solutions were prepared freshly before starting the experiment. Stock solution (1mg/ml) was prepared by dissolving 1g in 100 ml distilled water. Further samples were prepared by diluting the stock solution up to 20 ml solutions of four different concentrations (12.5, 25, 50, 100 mg/ml).

Anthelmintic assay

The anthelmintic assay was carried as per the method Ajaiyeoba et. al. [23]. with minor modifications [24,25]. Six groups of earthworms of approximately equal size were released in to 20 ml solutions of four different concentrations (12.5, 25, 50, 100 mg/ml) in petri dishes containing above solutions of extracts. Albendazole (MP Biomedicals, LLC, Solan, India) was used as reference standard and normal saline (0.9%v/v) as control. Time of paralysis and time of death of the worm were determined [26]. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water followed with fading away of their body colours [27].

RESULTS

From the preliminary phytochemical screening plant was found to be rich in saponin (highest in aqueous extract of stem bark), polyphenols (highest in aqueous extract of seed capsules and aqueous extract of stem bark), glycosides (highest in aqueous extract of seed capsules), tannins (highest in aqueous extract of stem bark), triterpenoids (highest in methanol extract of seed capsules) and flavonoids (highest in aqueous extract of seed capsules). The methanol and aqueous extracts of stem bark and seed capsules of the plant showed anthelmintic activity in dose dependent manner. The shortest time required for paralysis and death of earthworms was observed with 100 mg/ml of aqueous extracts of seed capsules as 9.98 ± 0.037 minutes and 16.02 ± 0.042 minutes, respectively followed by methanol extracts of seed capsules which showed paralysis and death time as 12.01 ± 0.033 min and 19.01 ± 0.047 min respectively at the same concentration. The same concentration of methanolic extracts of stem bark showed paralysis and death time as 16.06 ± 0.030 and 23.02 ± 0.048 minutes respectively, and followed by 21.04 ± 0.031 and 25.05 ± 0.039 minutes with 100 mg/ml aqueous extract of the stem bark. The paralysis time with standard albendazole was 8.67 ± 0.033 minutes and death time was 16.67 ± 0.02 minutes. In the present study, anthelmintic activity has been confirmed in the methanol and aqueous extracts of *Callistemon lanceolatus* (Sm.) Sweet stem bark and seed capsules at the concentrations of 12.5, 25, 50, and 100 mg/ml (Table.1, Figure 1-4). The values were

expressed as mean $\pm$ standard error of mean (S.E.M.) and statistical analysis was carried out by using one-way analysis of variance (ANOVA) method followed by Dunnett's test, at 1% level of significance (P-value < 0.01).

Table 1: Anthelmintic activity of methanolic and aqueous extracts of stem bark and seed capsules of *Callistemon lanceolatus* (Sm.) Sweet

Treatment	Concentration	Paralysis time(minutes)	Death time(minutes)
Control	--	--	--
Albendazole(Standard)	20mg/ml	8.67 $\pm$ 0.033***	16.67 $\pm$ 0.020**
Seedcapsules(Methanolicextract)	12.5mg/ml	74.76 $\pm$ 0.037**	92.01 $\pm$ 0.028**
	25mg/ml	72.99 $\pm$ 0.072*	81.84 $\pm$ 0.039***
	50mg/ml	18.00 $\pm$ 0.091**	29.00 $\pm$ 0.040***
	100mg/ml	12.01 $\pm$ 0.033**	19.01 $\pm$ 0.047***
Seed capsules(Aqueous extract)	12.5mg/ml	74.01 $\pm$ 0.063**	86.01 $\pm$ 0.039**
	25mg/ml	70.02 $\pm$ 0.025***	81.00 $\pm$ 0.045***
	50mg/ml	14.00 $\pm$ 0.039***	26.00 $\pm$ 0.054***
	100mg/ml	9.98 $\pm$ 0.037***	16.02 $\pm$ 0.042***
Stem bark(Methanolic extract)	12.5mg/ml	87.05 $\pm$ 0.037*	96.00 $\pm$ 0.046***
	25mg/ml	70.07 $\pm$ 0.036**	81.98 $\pm$ 0.044***
	50mg/ml	37.03 $\pm$ 0.032***	46.06 $\pm$ 0.055***
	100mg/ml	16.06 $\pm$ 0.030***	23.02 $\pm$ 0.048***
Stem bark(Aqueous extract)	12.5 mg/ml	95.04 $\pm$ 0.033**	105.90 $\pm$ 0.056**
	25mg/ml	88.02 $\pm$ 0.034***	79.03 $\pm$ 0.038***
	50mg/ml	38.03 $\pm$ 0.036***	49.00 $\pm$ 0.074***
	100mg/ml	21.04 $\pm$ 0.031***	25.05 $\pm$ 0.039***

Values are expressed as MEAN $\pm$ SEM, One way ANOVA followed by Dunnett's test. Here, n=6 in each group. *P<0.01=Significant, **P<0.01=More significant, ***P<0.0001=Extreme significant. Values P>0.05 is considered non-significant

Figure 1:Paralysis and death time in methanol extract of seed capsules of *Callistemon lanceolatus*(Sm.)Sweet

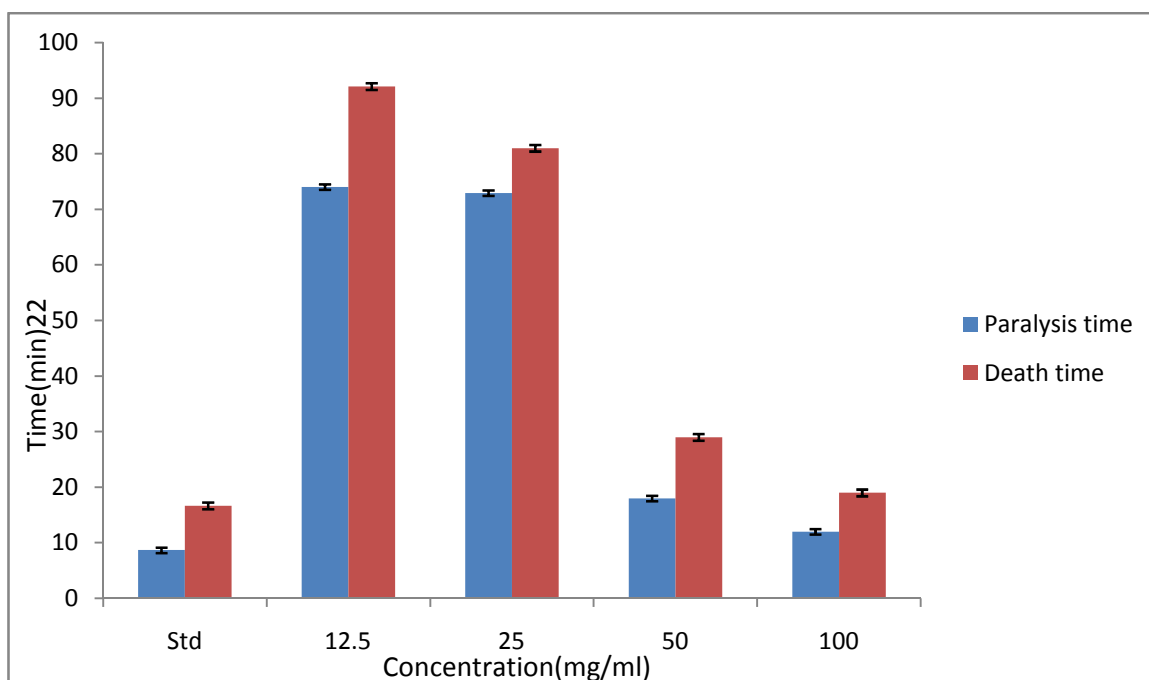


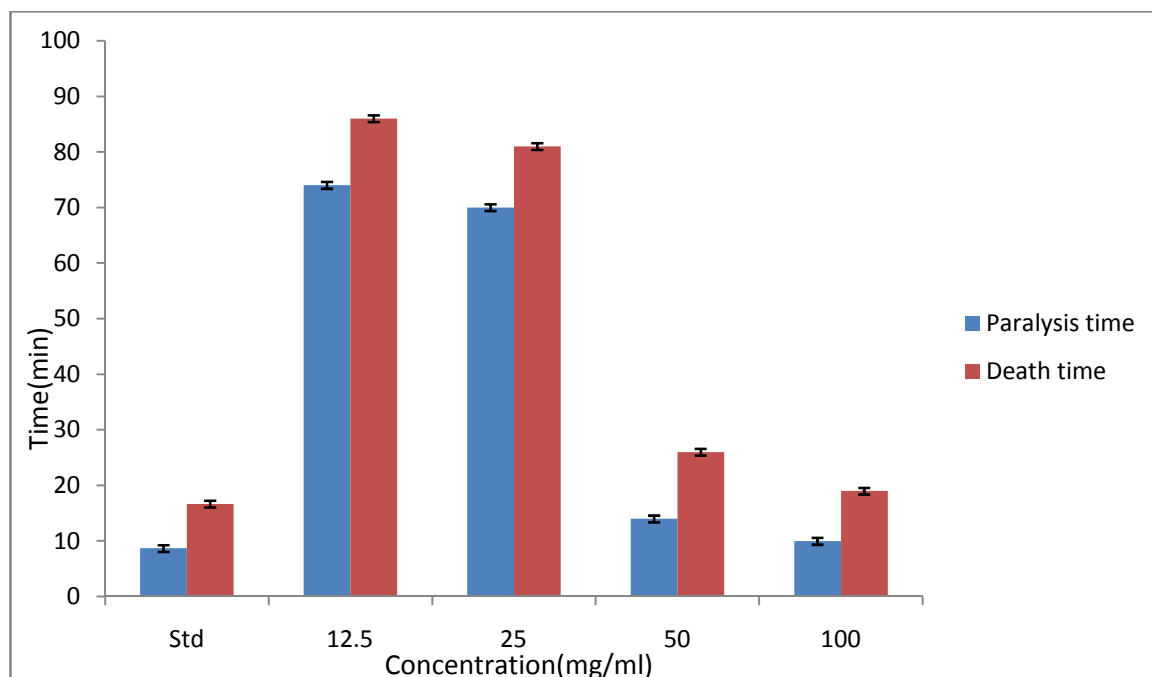
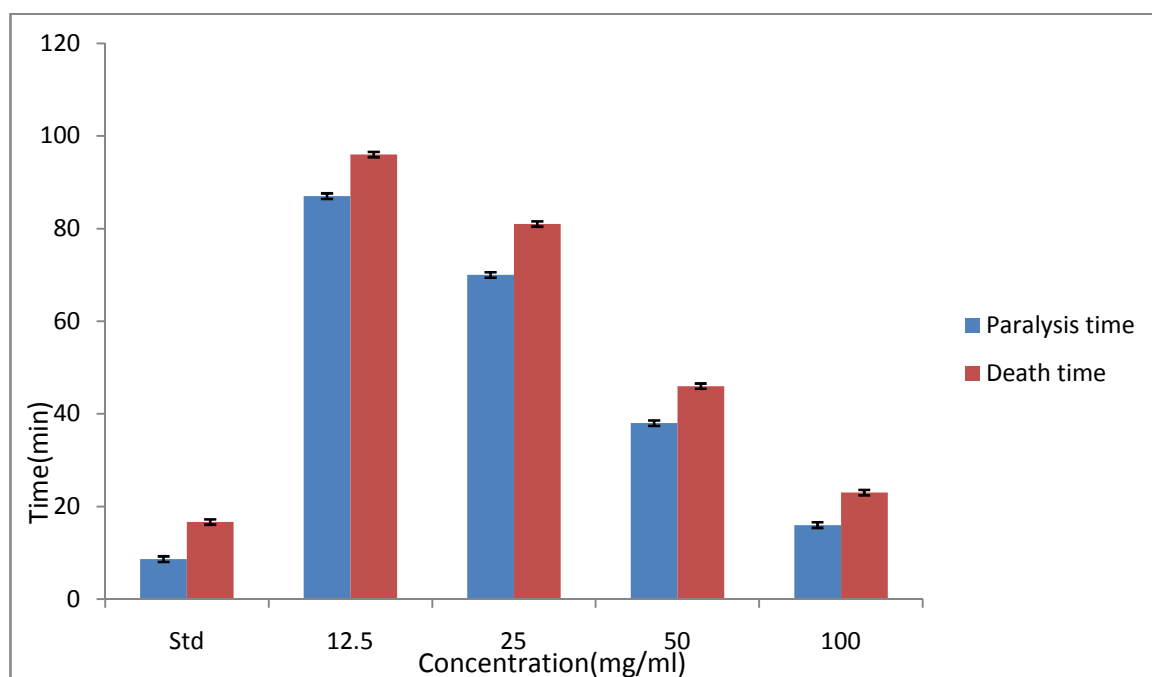
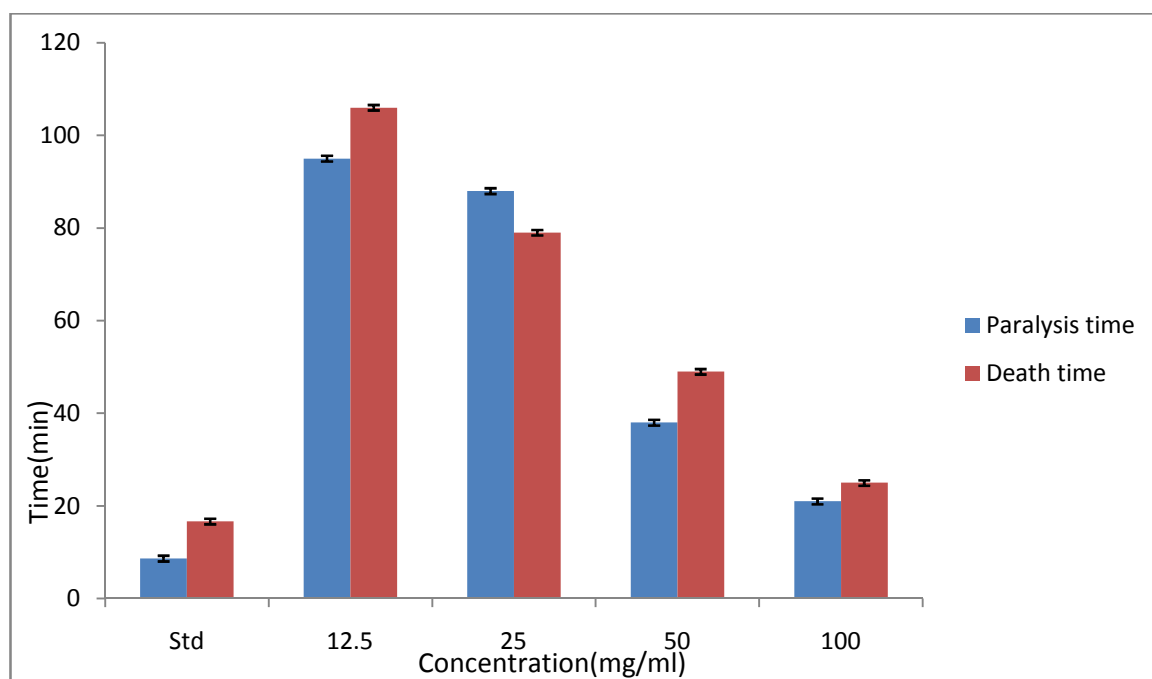
Figure 2: Paralysis and death time in aqueous extract of seed capsules of *Callistemon lanceolatus*(Sm.)SweetFigure 3: Paralysis and death time in methanol extract of stem bark of *Callistemon lanceolatus*(Sm.)Sweet

Figure 4: Paralysis and death time in aqueous extract of stem bark of *Callistemon lanceolatus*(Sm.)Sweet

DISCUSSION

The aqueous extract of seed capsules showed most significant anthelmintic activity, followed by the methanol extract of seed capsules. The methanolic extract of the stem bark showed higher activity than the aqueous extract. The least activity was shown by aqueous extract of the stem bark. The lethal effect of albendazole was attributed to its inhibition of tubulin polymerization and blocking glucose uptake. Polyphenolic compound, tannins, flavonoids and saponins are reported to be responsible for anthelmintic activity [28,]. The presence of polyphenols, tannins and saponins in the crude extracts of stem bark and seed capsules, as indicated by the preliminary phytochemical screening may be responsible for the anthelmintic activity. Thus strong anthelmintic activity of extracts under study is rationale with regards to its chemical constituents.

CONCLUSION

In conclusion, the present study provides evidence that both aqueous and methanol extract of seed capsules of *Callistemon lanceolatus*(Sm.)Sweet exhibited higher *in-vitro* anthelmintic activity on *Pheretima posthuma* than stem bark extracts. Although both parts of this plant showed anthelmintic activity therefore it can be plant of choice for helminthiasis.

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