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Phytochemical and Anthelmintic Studies on the Whole Plant of *Leonotis Nepetifolia* (L). R.BR.

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ABSTRACT

In the present study ethanolic and aqueous extracts of the whole plant of *Leonotis nepetifolia* (L). R.Br. were investigated for their anthelmintic activity against *Pheretima posthuma.* Three concentrations (25, 50 and 75 mg/ml) of each extract were subjected for evaluation. The study is mainly concerned with the determination of time of paralysis and time of death of the worm. The gradual increase in the dose showed a gradual increase in the anthelmintic activity. The ethanolic extract of the whole plant showed a significant anthelmintic activity at highest concentration of 75 mg/ml in shorter time as near to Albendazole (Standard drug).

Keywords: *Leonotis nepetifolia* (L). R.Br., *Pheretima posthuma*, Albendazole, Anthelmintic activity

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INTRODUCTION

Leonotis nepetifolia (L). R.Br. called Thenthumbai[1] in Tamil, belonging to the family Lamiaceae. Annual lion's ear is an erect, loosely branched annual that can get 8 ft (2.4 m) tall in its single growing season. The stems are strongly angled (square in cross section) and the leaves are in pairs opposite each other. The leaves are smooth with coarsely toothed margins, triangular in shape and 2-5 in (5.1-12.7 cm) long. The flowers are borne in rounded, spiny clusters, 2-4 in (5.1-10.2 cm) across, that encircle the stems so that it looks like the stems are growing right through the middle of the clusters. As the stems elongate, new flower clusters continue to develop above the older ones. The tubular flowers that peek out of the spiny heads are orange and furry, like a lion's ear. The flowers are about 1 in (2.5 cm) long and curve downward [2]. The plant is widely distributed in Pan tropics and is represented by 1 species - *Leonotis nepetifolia* (L). R.Br. in India with bright orange scarlet flowers. It is originally native to tropical and subtropical Africa. Kokani tribals of Nasik region of India region has been utilizing the paste of inflorescence of the plant with groundnut oil for wound healing [3 & 4]. The root paste and flower ash of this plant recommended for breast inflammation. The ethanolic extract of whole plant *Leonotis nepetifolia* recommended for antidiabetic activity in alloxan induced diabetic rats [5]. Plant flower juice with sugar as a cure of night blindness has been described in ethno pharmaceutical claims by the Vanrjari of Maharashtra State, India [6]. The plant inflorescence ash mixed with mustard oil for the post natal breast pain as followed and prescribed by the Tribal's of Kota Hills in Andhra Pradesh, India [7]. The plant flowers and seed paste with ghee for controlling cough and curing cuts, wounds and burns has also been reported [8]. The treatment of eczema by leaf paste of *Leonotis nepetifolia* (L). R.Br. and the leaf juice from *Leonotis nepetifolia* (L). R.Br. for curing malaria [9, 10 & 11].

MATERIALS AND METHOD

Plant Material

The whole plant of *Leonotis nepetifolia* (L). R.Br. (Figure 1) was collected from the Tirunelveli, Tamilnadu in the month of May 2016. The plant was then authenticated by the Dr. V. Chelladurai, M.Sc., Ph.D., Research officer botanist (Scientist - C), Central Council for Research in Ayurveda and Siddha, Govt. of India (Retired), Tirunelveli, Tamilnadu, India. The whole plant material were dried in shade and pulverized. The powder were passed through sieve no. 40 and used for the extraction.

Figure 1: *Leonotis nepetifolia* (L). R.Br.

Preparation of extracts [12]

Twenty gram of powdered plant material was kept in 200 mL conical flask and added 100 mL of solvent such as ethanol and water individually. The mouth of the conical flask was covered with aluminium foil and kept in a reciprocating shaker for 24 h for continuous agitation at 150 rev/min for thorough mixing and also complete elucidation of active materials to dissolve in the respective solvent. Then, extract was filtered by using muslin cloth followed by Whatman no. 1 filter paper. The solvent from the extract was removed by using water bath temperature of 50°C. Finally, the residues were collected and used for the experiment.

Phytochemical screening

Phytochemical screening of ethanolic and aqueous extracts of whole plant of *Leonotis nepetifolia* (L). R.Br. revealed the presence of glycosides, tannins & phenolics, flavonoids and triterpenoids. The results showed in Table 1.

Selection of worms

Adult Indian earthworms, *Pheretima posthuma* due to its anatomical and physiological resemblance with that of intestinal round worm parasite of human beings [13, 14, 15 & 16]. The earthworms were collected from moist soil and washed with normal saline to remove all faecal matter and were used for the anthelmintic study. The earthworms of 6-8 cm in length and 0.2-0.3 cm in width were used.

Experimental

The anthelmintic assay was carried as per the method followed by Ajaiyeoba et al[17] with minor modifications. The assay was performed on adult Indian earthworms, *Pheretima posthuma* due to its anatomical and physiological resemblance with that of intestinal round worm parasite of

human beings. Both extracts and standard drug solution were freshly prepared in normal saline before starting the experiments. Both extracts and standard drug solutions were poured in different petri plates. All the earthworms were released into 10ml of formulation as follows: ethanolic extract, aqueous extract and Albendazole in three different concentrations. Observations were made for the time taken to paralysis and death of worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility when dipped in warm water (50^{o}C) followed with fading away of their body colours.

Statistical Analysis

The statistical analysis were done by using Dunnett's test.

RESULTS AND DISCUSSION

Table 1:Indicates phytoconstituent present in ethanolic and aqueous extracts of whole plant of *Leonotis nepetifolia* (L). R.Br.

S.No.	Phytoconstituents	Inference	
		Ethanolic extract	Aqueous extract
1.	Glycosides	+	+
2.	Tannins & phenolics	+	+
3.	Flavonoids	+	-
4.	Triterpenoids	+	-

Table 2: Anthelmintic activity of ethanolic and aqueous extracts of whole plant of *Leonotis nepetifolia* (L). R.Br.

Treatment	Conc.(mg/ml)	Paralysis time (min)	Death time (min)
Control	-	-	-
Albendazole (Standard)	25	108±0.31	121±0.38
	50	64±0.73	81±0.12
	75	45±0.25	61±0.26
Ethanolic extract	25	112±0.25	132±0.14
	50	72±0.43	90±0.31
	75	52±0.56	69±0.29
Aqueous extract	25	118±0.20	139±0.33
	50	79±0.28	98±0.10
	75	56±0.32	72±0.27

Values are mean ± SEM, N=3**P<0.01 compared with the standard

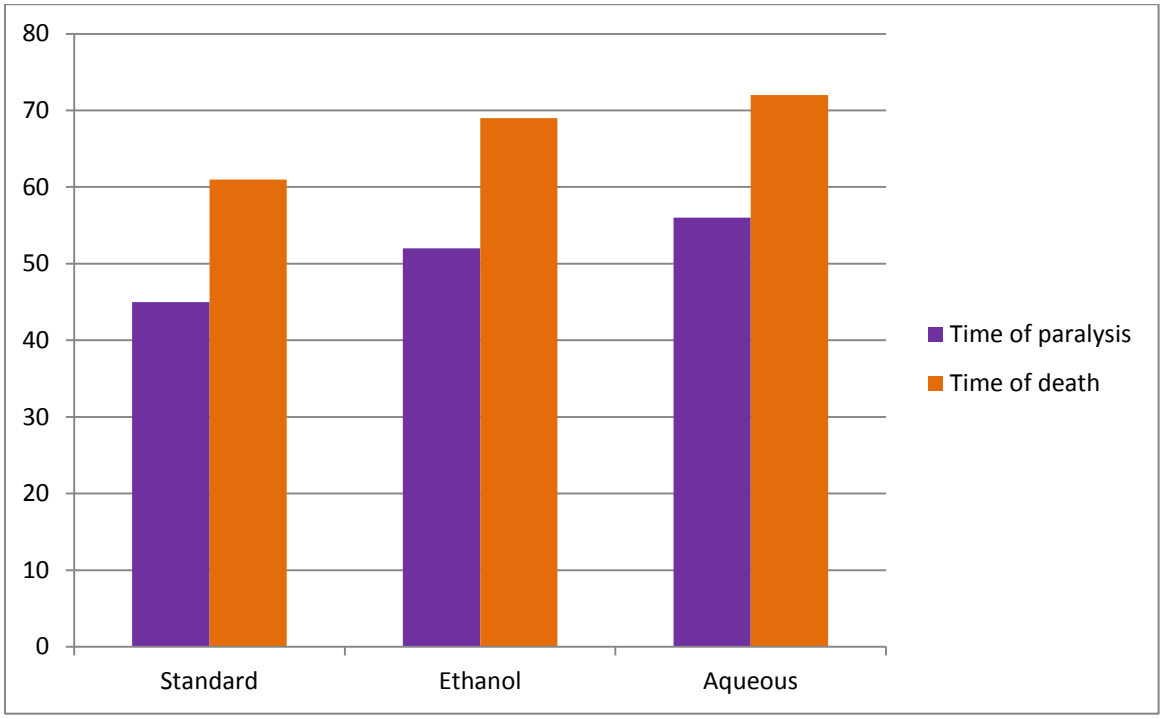

Figure 2: Anthelmintic activity of ethanolic and aqueous extracts of whole plant of *Leonotis nepetifolia* (L). R.Br.

The detailed literature survey of the plant reveals its usage among the tribal's in treating intestinal worm infections. The data obtained in the study could provide a rationale for the traditional use of this plant as an anthelmintic. Phytochemical screening of the extracts revealed the presence of glycosides, tannins & phenolics, flavonoids and triterpenoids. As per literature survey, tannins chemically polyphenolic compounds[18], were shown to produce anthelmintic activities[19]. Reported anthelmintic effect of tannins, can bind to free proteins in the gastrointestinal tract of host animal[20] or glycoprotein on the cuticle of the parasite and may cause death.The ethanolic and aqueous extracts of the plant displays a significant anthelmintic activity in dose dependent manner (Table 2 & Figure 2). The anthelmintic activity of these extracts were comparable with that of standard drug. The probable mechanism of action of the whole plant of *Leonotis nepetifolia* (L). R.Br. as an anthelmintic could be due to its tannins chemically polyphenolic compounds.

CONCLUSION

It is concluded based on the findings of the present study, that the anthelmintic activity of the extracts is dose dependent. The ethanolic and aqueous extracts exhibited significant anthelmintic activity in *Leonotis nepetifolia* (L). R.Br. at highest concentration of 75 mg/ml (Table 2 & Figure 2). This study strongly supports the traditional use of this plant as an anthelmintic.

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