



***In vitro* anthelmintic activity of *Leonotis nepetiifolia* (L.) R.Br., a potential medicinal plant**

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ABSTRACT

*The present paper deals with anthelmintic activity of leaves of *Leonotis nepetiifolia* (L.) R.Br. (Lamiaceae), a potential medicinal plant, distributed in wastelands, open forests in India. The plant is used in traditional medicine for bronchial asthma, diarrhoea, fever, cold, cough, malaria and as an analgesic agent in menstrual pains. The methanol, ethyl acetate and aqueous extracts were evaluated for its anthelmintic activity against adult indian earth worms (*Phertima posthuma*). Three concentrations (25, 50 and 75mg/ml) of each extract were studied, which involved for the determination of time of paralysis and time of death of the test worms. It was found that methanol and ethyl acetate extracts exhibited significant anthelmintic activity while aqueous extract show least activity. Albendazole in same concentration as that of extract was used as standard reference and saline water as control.*

Key words: *Leonotis nepetiifolia*, Anthelmintic activity, *pheritima posthuma*, Albendazole.

INTRODUCTION

Helminthiasis is a world wide and one of the common diseases of all ages especially in third world countries. The parasitic diseases cause severe morbidity by affecting population in endemic areas with major economic and social consequences [1]. Helminth infections are among the most common infections in man, affecting a large proportion of the world's population. In developing countries they pose a large threat to public health and contribute to the prevalence of malnutrition, anaemia, eosinophilia, and pneumonia. Although the majority of infections due to worms are generally limited to tropical regions, they can occur to travellers who have visited those areas and some of them can develop in temperate climates [2]. Helminthiasis is a disease in which a part of the body is infested with worms such as pinworm, roundworm or tapeworm. Typically, the worms reside in the gastrointestinal tract but may also burrow into the liver and other organs, infected people excrete helminth eggs in their faeces, which then contaminate the soil in areas with inadequate sanitation [3]. Other people can then be infected by ingesting eggs or larvae in contaminated food, or through penetration of the skin by infective larvae in the soil (hookworms). Parasitic diseases cause severe morbidity, including filariasis (a cause of elephantiasis), onchocerciasis (river blindness), and schistosomiasis [4]. As per WHO only synthetic drugs are frequently used in the treatment of helminth infestations in human beings but these synthetic drugs are out of reach of millions of people and have a lot of side effect. In view of this, an attempt has been made to study the anthelmintic activity of herbal drug.

Leonotis nepetiifolia, called Hanumanthabira in Telugu (local language), belonging to the family Lamiaceae. The plant is used in the treatment of asthma in the Carribean [5]. It is also used in the treatment of rheumatism, rickets, diarrhoea, fever, malaria, analgesic agent in menstrual pains, headaches and for the treatment of wounds [6] by the local people. The present study deals with the anthelmintic effect of *Leonotis nepetiifolia* using a standard laboratory procedure.

EXPERIMENTAL SECTION

Plant material

The specimens were collected from the Chittoor district, Andhra Pradesh, identified with the help of regional and local floras [7,8] and the voucher specimens were deposited at Sri Krishnadevaraya University Herbarium (SKU), Anantapur. The plant material was washed with water and shade dried, pulverized in mechanical grinder and stored in an airtight container till further successive extractions.

Preparation of aqueous extract

The dried powder (70g) was extracted with water for 72hr and the same was dried on water bath [9].

Preparation of methanol and ethyl acetate extract

The dried powder (70g) extracted in a soxhlet apparatus using methanol (95%) and ethyl acetate, at a temperature range of 45°C to 60°C. The filtrate was evaporated to dryness at reduced pressure in vacuum evaporator [9].

Experimental procedure

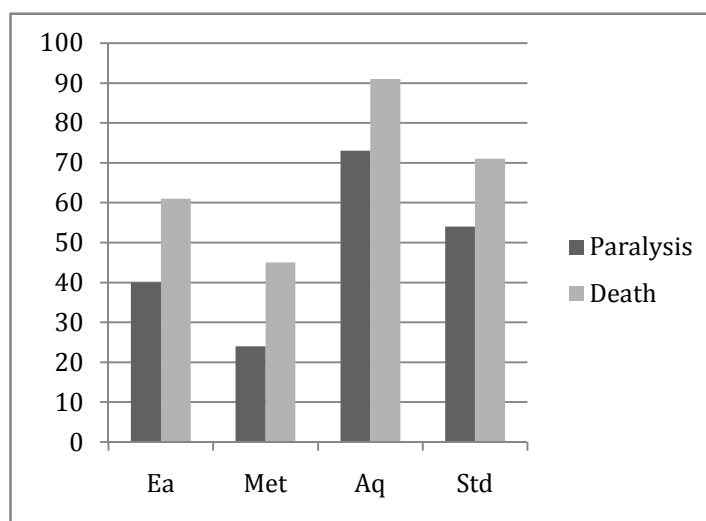
Methanol, ethyl acetate and aqueous extracts from the leaves of *Leonotis nepetiifolia* were investigated for anthelmintic activity against *Pheretima posthuma*. Various concentrations (25, 50 and 75 mg/ml) of each extract were tested by bioassay, which involved determination of time of paralysis and time of death of the worms. Albendazole was used as standard reference and saline water as control. The Anthelmintic assay was carried as per the method followed by Ajaiyeoba *et al* with minor modifications [10]. 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 [11, 12, 13]. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro* [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 for all experimental protocol. The earthworms were divided into thirteen groups containing six earthworms in each group. All the extracts and standard drug solution were freshly prepared in normal saline before starting the experiments. Different extracts and standard drug solutions were poured in different petri plates. All the earthworms were released into 10ml of formulation as follows: Methanol, ethyl acetate, 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°C) followed with fading away of their body colors.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis showed the presence of alkaloids, phenols, flavonoids, steroids and tannins like phytoconstituents in the extracts of *Leonotis nepetiifolia*. Some of these phytoconstituents may be responsible to show a potent anthelmintic activity. From the observations made all the extracts of whole plant of *Leonotis nepetiifolia* was found to show a potent anthelmintic activity when compared to the standard drug. Methanolic extract of at 75 mg/ml concentration shows paralysis at 24 min and death 45 min, whereas ethyl acetate extract shows paralysis at 40 min and death 61 min respectively while aqueous extracts show paralysis at 73 min and death 91 min by the earth worm *Pheretima posthuma*. Among the three extracts aqueous extracts show least anthelmintic activity. The reference drug Albendazole exhibited the same at 54 min and 71 min respectively [Table1, Fig1]. Albendazole exhibits anthelmintic activity by blocking glucose uptake and depletion of glycogen stores in test parasite. The methanol and ethyl acetate extracts of *Leonotis nepetiifolia* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 75 mg/ml in shorter time as compared to that of Albendazole. Phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, tannins and steroids. Tannins chemically polyphenolic compounds [18], were shown to produce anthelmintic activities [17]. Reported anthelmintic effect of tannins, can bind to free proteins in the gastrointestinal tract of host animal [19] or glycoprotein on the cuticle of the parasite and may cause death. Further studies are under process to identify the possible phytoconstituents responsible for anthelmintic activity.

Table -1:Anthelmintic activity of the extracts of *Leonotis nepetifolia*

Treatment	Conc (mg/ml)	Paralysis time (min)	Death time (min)
Ethyl acetate	25	87	106
	50	52	69
	75	40	61
Methanol	25	68	82
	50	37	56
	75	24	45
Aqueous	25	112	140
	50	84	101
	75	73	91
Albendazole	25	110	134
	50	68	87
	75	54	71
Control	-	-	-

Fig -1:Anthelmintic activity of various extracts of *Leonotis nepetifolia*

CONCLUSION

The traditional use of the leaves of *Leonotis nepetifolia* as anthelmintic has been confirmed using the different extracts and showed significant anthelmintic activity. Further it would be interesting to isolate the responsible phytoconstituents, which are responsible for the anthelmintic activity and the mechanism of action, which is being attempted in the laboratory.

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