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In vitro evaluation of anthelminthic activity of *Ludwegia* perennis leaf extracts

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Abstract
The purpose of this investigation was to evaluate the anthelminthic activity of *Ludwegia* perennis leaf extracts.. The leaves were made free from dust and foreign material and dried powdered and extracted by successive solvent extraction using different solvents like petroleum ether, n-hexane, ethanol and water. Phytochemical screening was carried out for the detection of phytoconstituents. The paralysis and death time were determined at the concentrations of 25mg/ml, 50mg/ml and 100mg/ml. The extracts of the *Ludwegia* perennis leaves have shown significant anthelminthic activity at concentrations of 25mg/ml, 50mg/ml and 100mg/ml compared with Albendazole as standard drug. The results from the study conclude that the Petroleum ether extract of *Ludwegia* perennis leaves exhibit more potent anthelmintic effect while the other extracts showed slightly less activity compared to that of Albendazole.

Keywords: *Ludwegia* perennis, anthelminthic, phytochemical, albendazole

Introduction
Helminths are a broad range of organisms that include intestinal parasitic worms, (roundworms, Ascaris lumbricoides), whipworms (Trichuris trichiura), or hookworms (Necator americanus and Ancylostoma duodenale). The word helminth is derived from the Greek word helmins which means parasitic worm. Helminths are highly prevalent and, depending on the species, may exit as free-living organisms or as parasites of plant or animal hosts. Helminthiasis is also called worm infection and is a macro parasitic disease occurring in mankind and animals. Helminthic infections are among the most common infections in human beings affecting a large population of the world. Helminthiasis is among the most important diseases inflicting heavy production losses. Helminths are the most common disease causing agents of humans in developing countries and produce a global burden of disease and contribute to prevalence of malnutrition, anaemia, eosinophilia, and pneumonia [1]. The disease is highly prevalent particularly in third world countries due to poor management practices. However, increasing problems of development of resistance in helminths [2, 3] against anthelmintics have led to proposal of screening medicinal plants for their anthelmintic activity. The plants are known to provide a rich source of botanical anthelmintics [4, 5]. Nowadays the medicinal preparations available in market are either not effective up to the mark or develop resistance resulting in reoccurrence again. Plant derived drugs serve as a prototype to develop more effective and less toxic medicines. Anthelmintics are drugs that are used to treat infections with parasitic worms. This includes both flat worms, e.g., flukes and tapeworms and round worms, i.e., nematodes. They are of huge importance for human tropical medicine and for veterinary medicine [6]. The World Health Organization estimates that a staggering 2 billion people harbour parasitic worm infections. Parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact. Also of importance is the infection of domestic pets. Indeed, the companion animal market is a major economic consideration for animal health undertaking drug programmes. Despite the prevalence of parasitic worms, anthelmintic drug discovery is the poor relation of the pharmaceutical industry. The simple reason is that the nations which suffer most from these tropical diseases have little money to invest in drug discovery or therapy. It comes as no surprise therefore that the drugs available for human treatment were first developed as veterinary medicines. This prompts concern, as anthelmintic resistance has been widely reported in livestock and it may also only been a matter of time before this phenomenon occurs in parasites of humans [7]. Anthelmintics are a group of antiparasitic drugs that expel parasitic worms (helminths) and other internal parasites from the body by either stunning or killing them and without causing significant damage to the host. They may also be called vermifuges (those that stun) or vermicides (those that kill). Helminthic infection is among the most common infections in human beings, affecting a large population of the world's population [8]. The search for new bioactive agents led to the screening for bioactive compounds in *Ludwigia* perennis. The genus *Ludwigia* perennis is composed of about 34 species. Wide phytochemical studies have reported that *Ludwigia* genus is rich in alkaloids, glycosides, tannins, flavonoids and free sugars on phytochemical screening [9]. It belongs to family Onagracease and is native to India and East indies. It is found throughout India upto an altitude of 600mtrs and also found in other countries like Bangladesh, Combodia, Srilanka, Thailand, Vietnam and Mizoram. Traditionally the leaves of the plants are used in the treatment of rheumatism and other ailments [10]. However literature survey has no scientific data on anthelminthic activity on *Ludwigia* perennis.

Material and Methods
Collection of plant material
The plant material was collected from Hyderabad and was authenticated by Osmania University, Hyderabad and was given the Voucher no 134.

Drugs and chemicals

Albendazole was procured from Abbott health care Pvt.Ltd. Petroleum ether, methanol were procured from SD, Fine chemicals, Reagents for phytochemical screening were prepared freshly in the drug store of Anwarul uloom College of Pharmacy.

Methods

Extraction process

The plant material was dried under shade and was finely powdered, pulverized by a mechanical grinder passed through a #40 sieve and stored in tightly closed container for further use.The dry powder was extracted with methanol, petroleum ether, n-hexane and aqueous solvent using process of Soxhlet extraction [11].

Phytochemical screening

Preliminary phytochemical screening was done to identify the presence of alkaloids, steroids, flavonoids and tannins in leaf extracts. The preliminary

Phytochemical screening of extracts was performed by the method described by Khandelwal [12].

Evaluation of Anthelminthic activity

The anthelminthic assay was carried out by the method of Ajaiyeobaet,al[13].Adult earthworms of about 3-6 cm in length were used to evaluate anthelminthic activity *in vitro*. Earthworms were washed with saline to remove dirt. Test samples of the extracts of methanol, petroleum ether was prepared at concentrations of 25, 50, 100 mg/ml in distilled water. Albendazole (25mg/ml) was used as standard drug. Observations were noted for the time taken for paralysis and death of individual worms. Time for paralysis was taken when no movement or any sort could be observed except when the worms were shaken vigorously. Time for death of worms were observed after ascertaining that the worms neither moved when shaken nor when dipped in warm water at 500 C followed with fading of their body colour [14].

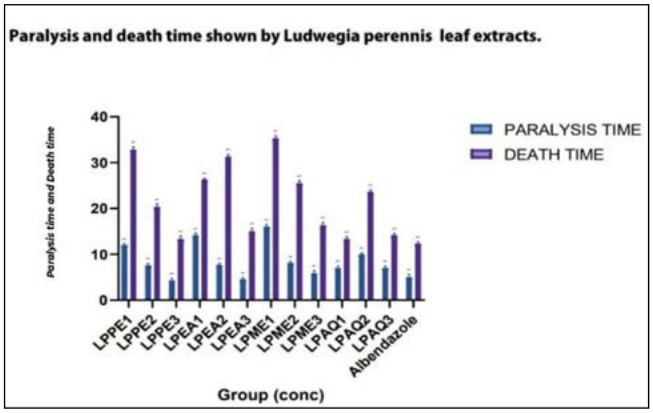
Results and Discussion

Results

In the first experimental group (Albendazole) at 50mg/ml concentration the paralysis time was found to be (5±0.653) and the death time was (12.33±0.421). In the second experimental group (Petroleum extract) at 25mg/ml concentration the paralysis time was (12±0.365) and the death time was (32.83±0.600). Increase in concentration of 50 mg/ml the paralysis time was (7.6±0.416) and the death time (20.33±0.701). At highest concentration of 100mg/ml the paralysis time was (4.33±0.421) and the death time was (13.33±0.667).In the third experimental group (Ethyl acetate extract) at 25mg/ml concentration the paralysis time was found to be (14.16±0.466) and the death time was found to be (26.33±0.333). Increase in the concentration of 50mg/ml the paralysis time was found to be (7.66±0.421) and the death time was (31.33±0.494). At highest concentration of 100mg/ml the paralysis time was (4.5±0.428) and death time was (15±0.638). In fourth experimental group (Methanol extract) at 25mg/ml concentration the paralysis time and death time were found as (16±0.577) and (35.33±0.557) respectively. At 50mg/ml the paralysis time was (8.16±0.307) and death time was (25.5±0.562).At 100mg/ml the paralysis time was (5.83±0.577) and death time was (16.33±0.616).In the fifth experimental group (Aqueous extract) at 25mg/ml concentration the paralysis time was (7±0.447) and death time was (13.33±0.494). Increase in concentration of 50mg/ml the paralysis time was to be (10 ±0.365) and the death time was (23.66±0.421).At 100mg/ml concentration the paralysis time was (7±0.447) and death time was (14.16±0.421) as depicted in Table 1 and Graph 1.

Table 1: Table depicting Anthelmintic activity of extracts at different concentrations.

Sno	Group	Concentration	Paralysis time	Death time
1	Albendazole			
		50mg/ml	5±0.653	12.33±0.421
2	LPPE	25mg/ml	12±0.365	32.83±0.600
		50mg/ml	7.6±0.416	20.33±0.701
		100mg/ml	4.33±0.421	13.33±0.667
3	LPEA	25mg/ml	14.16±0.466	26.33±0.333
		50mg/ml	7.66±0.421	31.33±0.494
		100mg/ml	4.5±0.428	15±0.638
4	LPME	25mg/ml	16±0.577	35.33±0.557
		50mg/ml	8.16±0.307	25.5±0.562
		100mg/ml	5.83±0.577	16.33±0.616
5	LPAQ	25mg/ml	7±0.447	13.33±0.494
		50mg/ml	10±0365.	23.66±0.421
		100mg/ml	7±0.447	14.16±0.421



Graph 1

Discussion

There have been many advancements made and many drugs were developed for treatment of the damage caused by the helminthic parasites. But there is no effective medicine developed so far. The serious side effects of drugs and development of resistance has derived the severity of infection to higher level. Preliminary phytochemical screening of extracts of leaves of *Ludwegia* perennis have shown the presence of alkaloids, flavonoids, tannins and saponins. Tannins and polyphenolic compounds were shown to produce anthelmintic activities. The triterpenoids may inhibit the food intake and cause paralysis and death of the organisms. The anthelmintic activity was conducted with Petroleum ether, Ethyl acetate, Methanol and leaf extracts of *Ludwigia* perennis at doses of 25, 50 100mg/ml. The ethanolic leaf extract of *Ludwigia* perennis showed the death of worms in less time as compared to that of albendazole at higher concentration of 100mg/ml. Methanolic extracts produced paralysis ranging from loss of motility to loss of response

to external stimuli in a significant manner ($p<0.01$). This plant may be further recognised for its phytochemical

profile for the active constituents for Anthelmintic activity. The effect of albendazole on worms is to cause paralysis that result in expulsion of worm by peristalsis. Albendazole acts by increasing chloride ion conductance of worm muscle membrane producing hyperpolarization and reduced excitability leading to muscle relaxation and flaccid paralysis [15].

Conclusion

Ludwigia perennis has shown significant anthelmintic activity. Further isolation and characterization of bioactive compounds from the plant is in progress.

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