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In-Vivo Anthelmenthic Activity of Various Extracts of *Eclipta Alba* Leaves

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Abstract:

Different extracts of *E.alba* were taken for anthelmintic activity against Indian earthworm Pheretima posthuma. Two concentrations (100 and 150 mg/ml) of various extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. Albendazole (20 mg/ml) was used as reference standard. Dose dependent activity was observed in the plant extracts but ethanolic extract exhibited more activity as compared to others.

1. Introduction

Helminths are parasitic worms. They are the most common infectious agents of humans in developing countries and produce a global burden of disease that exceeds better-known conditions, including malaria and tuberculosis (Peter JH et al., 2008). Helminthiasis is a disease in which a part of the body is infested with worms such as pinworm, roundworm or tapeworm it is the most prevalent disease and amongst the most serious problems in the world. 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 (IK. Idika., 2012). Helminthiasis contributes to malnutrition anemia, eosinophilia and pneumonia (D. Anantha et al., 2010, Choudhury Golak. As per WHO only synthetic drugs are frequently used in the treatment of helminth infestations in humans but these synthetic drugs are out of reach of many people and have a lot of side effects. In view of this, an attempt has been made to study the anthelmintic activity of herbal drug.

Eclipta alba (L.) belong to the family Asteraceae commonly called as Bhringaraj in India, that is of herbaceous type and spreads on ground or partly ascending with its stem, small leaves are succulent and are found mostly in tropical and subtropical regions where water logging condition is very high. The medicinal properties of plants such as hepatoprotective, antimicrobial, anti-inflammatory, analgesic, immunomodulatory, antiviral, promoter for blackening, growth of hair (V.M. Jadhav et al., 209) and anti-ulcer activity (Banerjee A et al., 2005) have been investigated in the recent scientific developments throughout the world, due to its potent antioxidant activities (T. Regupathi et al., 2015), no side effects and economic viability (Karthikumar, S, K et al., 2007). Keeping in mind such astounding properties exhibited by the plant, the present study was intended to investigate anthelmintic activity of chloroform, ethanolic and aqueous extract of *Eclipta alba* leaves in Indian earthworm.

2. Material and method

2.1 Plant material

The leaves of *E.alba* were collected in the month of August locally from Raigir village, District Yadadri of Telangana state, India and were authentified by Dr. Ram Mohan, Taxonomist, AGI as *E. alba* leaves.

2.2 Experimental worms

All the experiments were carried out in Indian adult earthworms (Pheretima posthuma) due to its anatomical resemblance with the intestinal roundworm parasites of human beings. They were collected from moist soil and washed with water to remove all faecal matters.

2.3 Drugs and Chemicals

Albendazole, ethanol, chloroform were purchased from the UV scientifics private limited. All the chemicals used are laboratory and analytical grade.

2.4 Preparation of Extracts (Kokate CK., 2008)

2.4.1 Preparation of chloroform extract

The fresh leaves were dried under shade. The dried leaves were cut into small pieces and powdered in a hand mill. Then 300 g of coarse powder was extracted with 900 ml of extra pure chloroform and (WHO recommended) successively in a soxhlet extractor repeatedly for 48 hours. The extract was dried by solvent

evaporation in a thermostat water bath at 50-60^0 C temperature.

2.4.2 Preparation of aqueous extract

300 g of coarse powder of *E. alba* leaves was macerated with 900 ml of distilled water for 48 hours. After completion of 48 hours it is filtered to separate the water extract from the marc. The extract was concentrated in a thermostat water bath at 70-80^{0}C temperature and all the extracts were kept in dessicator for the experiment.

2.4.3 Preparation of ethanol extract

Another 30 g of coarse powder of *E. alba* leaves was soaked in 200 ml of ethanol for 48 hours with occasional ultrasonication by setting a duration of every 5 minutes for period of 30 minutes to prevent heating in the ultrasonicator bath with the continuous operation. The extract was passed through Whatman filter paper No. 1. It was concentrated by rotary evaporator setting 40^{0}C for 40 minutes. 500 mg of the crude extract was weighed in a dry clean vial and was used for further works (Ahmad. I et al., 2007).

2.5 Preliminary Phyto-chemical Investigation

The percentage yield of chloroform, ethanol and aqueous extract obtained was calculated and all the extracts were subjected to number of proximate qualitative phyto-chemical analysis. These procedures are already reported by number of workers and used without any modification (Nivedita et al., 2013).

2.6 Experimental Design (Ajaiyeoba EO et al., 2012, Kumar T et al., 2011, Kosalge SB et al., 2009, Chandan HS et al., 2012)

The anthelmintic activity was carried out as described by Ajaiyeoba EO et. al, 2001, with minor modifications. The Indian earthworm (*Pheretima posthuma*) of nearly equal size, six in each group was taken for the experiment. Each type of dried extract was suspended in 1% w/v carboxy methyl cellulose, prepared in normal saline in two different concentrations (100, 150 mg/ml). Albendazole suspension of 20 mg/ml concentration was taken as standard and normal saline water with 1% CMC was taken as a control. Worms were placed in petridish containing 15 ml of sample (drug) solution. Time for paralysis was noted either when any movement could not be observed except when the worms were shaken vigorously or when dipped in warm water (50^{0}C). Death was included when the worms lost their motility followed by white secretions and fading away of their body colour.

Table 1: Preliminary Phytochemical screening of *E.alba* leaves

S.No	Constituents	Ethanol	Water	Chloroform
1	Carbohydrates	-	+	-
2	Proteins	-	+	-
3	Anthraquinones	-	-	-
4	Flavonoids	+	+	+
5	Terpenoids	+	+	+
6	Saponins	+	+	-
7	Alkaloids	+	+	-
8	Tannins	+	+	-
9	Glycosides	+	-	-
10	Steroids	+	-	+

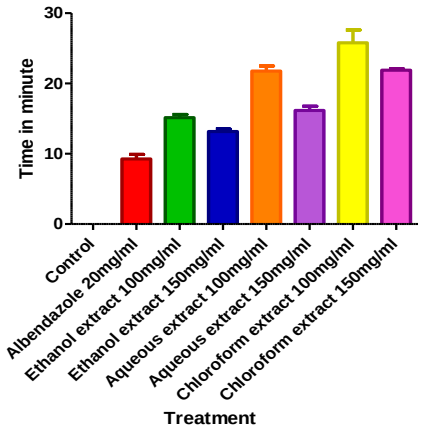

Figure 1: Paralytic effect of ethanolic, aqueous and chloroform extracts of *E.alba* on *Pheretima posthuma*

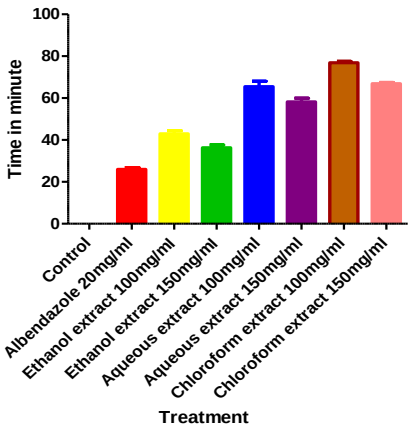

Figure 2: Effect of ethanolic, aqueous and chloroform extracts of *E.alba* on death of *Pheretima posthuma*

Table 2: Anthelmintic activity of various extracts of *Eclipta alba* Leaves

S.No	Treatment	Concentration (mg/ml)	*Pheretima posthuma*	
			Paralysis (P)	**Death (D)**
1	Control (0.5% CMC)	-	-	-
2	Standard (Albendazole)	20	9.267±1.102	25.94±1.084
3	Ethanol	100	15.13±0.778	42.93±2.702
		150	13.17±0.585	36.27±2.369
4	Aqueous	100	21.17±1.233	65.47±4.402
		150	16.17±1.012	58.17±3.066
5	Chloroform	100	25.80±3.158	76.87±1.150
		150	21.89±0.355	66.87±1.050

3. Results and discussion

Preliminary phytochemical analysis of ethanolic extracts showed the presence of flavonoids, saponins, tannins, steroids, terpenoids, glycosides & alkaloids as active phytoconstituents. Aqueous extracts showed the presence of carbohydrates, proteins, flavonoids, saponins, tannins, terpenoids, & alkaloid, where as chloroform extract showed the presence of flavonoids, terpenoids and steroids active phytoconstituents. The presence of phyto-constituents is reported in Table 1.

From this study it is revealed that all the extracts of *E.alba* showed anthelmenthic activity. When compared to the standard drug. Ethanolic extract at 100 mg/ml concentration shows paralysis at 15.13 min and death 42.93 min, where as aqueous extract shows paralysis at 21.17 min and death 65.47 min respectively while chloroform extracts show paralysis at 25.80 min and death 76.87 min by the earth worm *Pheretima posthuma*. Ethanolic extract of at 150 mg/ml concentration shows paralysis at 13.17 min and death 36.27 min, where as aqueous extract shows paralysis at 16.17 min and death 58.17 min respectively while chloroform extracts show paralysis at 21.89 min and death 66.87 min by the earth worm *Pheretima posthuma*. Among the three extracts chloroform extract shows least anthelmintic activity and ethanolic extract showed maximum activity comparatively. The reference drug albendazole exhibited the same at 9.267 min and 25.94 min respectively (Table 2, Figure 1 and Figure 2). Potency of the extracts was found to be inversely proportional to the time taken for paralysis/death of the worms.

4. Conclusion

It is revealed that the ethanolic, aqueous and chloroform extracts obtained from the leaves of *Eclipta alba* possess anthelmintic activity, but the ethanolic extract showed enhanced anthelmintic activity when compared with the aqueous and chloroform extract and standard drug, albendazole. The active constituents such as flavanoids, alkaloids and terpenoids might be responsible for anthelmintic activity because of their antioxidant property.

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