

**PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIHELMINTHIC ACTIVITY OF
BUTEA MONOSPERMA (L) BARK ETHANOLIC AND AQUEOUS EXTRACT**

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ABSTRACT

Ethanollic and aqueous extracts from the stem bark of *Butea monosperma* (L) were investigated for anthelmintic activity against *Pheretima posthuma*. Various concentrations (20-60 mg/ml) of each extract were used in the bioassay which involved the parameters such as time of paralysis and time of death of worms. Standard Ethanollic extract 40mg/ml has shown significant effect by taking less time to paralyze as well as death ($P<0.05$), similarly concentration of 60mg/ml has shown significant effect on time of paralysis 12 ± 0.683 ($P<0.01$) and time of death 13 ± 0.577 ($P<0.05$) when compared with standard Albendazole treated groups.

KEYWORDS: Ethanollic extract, aqueous extract, *Butea monosperma* (L), *Pheretima posthuma*, Albendazole.

INTRODUCTION

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, anemia, eosinophilia, and pneumonia. Although the majority of infections due to worms are generally limited to tropical regions, they can occur to travelers who have visited those areas and some of them can develop in temperate climates¹. Parasitic diseases cause severe morbidity, including lymphatic filariasis (a cause of elephantiasis), onchocerciasis (river blindness), and schistosomiasis. These infections can affect most populations in endemic areas with major economic and social consequences².

The use of herbs and medicinal plant as the first medicines is a universal phenomenon. Every culture on the earth, through written or oral tradition, has relied on the vast variety of natural chemistries found in plants for their therapeutic properties. All drugs from the plant are substances with a particular therapeutic action extracted from plants³. *Butea monosperma* (L) is a deciduous tree, belongs to family fabaceae, which grows up to 15 m in height and 1.5- 1.8m in girth, with a crooked trunk. Bark is light- brown or bluish grey, yielding a ruby-red vitreous gum. Wood white or yellowish-brown, often becoming grey or grayish- brown, leaves 3- foliolate, large, unequal, 10.2-20.4cm. Flowers are borne in racemes, brilliant orange red, 3.8-5.1cm long, lower calyx-teeth deltoid, pods silvery- white, broad dehiscing by one suture. Seeds are flat, elliptic, reddish- grey, 3.2cm⁴.

MATERIALS AND METHODS**Plant Material**

Bark of *Butea monosperma* plant was collected from local region of Narsapur, District of Medak, and Andhra Pradesh, India in the month of June, 2010. The botanical identity was confirmed by a botanist Prof T.Mohana Department of Botany, Government Mehubia Junior College, Gunfoundry, Hyderabad. (Reference No: 3/2010)

Preparation of Extracts

5 Kg of bark was crushed to coarse powder and passed through sieve # 44. The sieved powder was stored in air tight, high density poly ethylene containers before extraction. Extraction was performed by using soxhlet apparatus (12 hours), carried out first with petroleum ether (60-80°C) to defat the material. The defatted material was then extracted with ethanol to get ethanollic extract and finally with water and small quantity of Chloroform (as 6.5ml/litre) as preservative to get aqueous extract. The aqueous and ethanollic extracts were

concentrated for further studies at reduced pressure and temperature in a rotary evaporator and tested for presence of secondary metabolites by different phytochemical tests⁵.

Preliminary Phytochemical Analysis

The bark extract was screened for the phytochemical components using the standard Method^{5,6}. The phytochemical components analyzed were alkaloids, steroids, starch, proteins, anthraquinone glycosides, saponins, flavonoids, tannins, and cardiac glycosides (Table1).

Evaluation of anthelmintic activity

Pheretima posthuma is commonly known as earthworms were collected (due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being) from water logged area near Narsapur, Medak Dist, and Andhra Pradesh. Anthelmintic activity was carried out on adult earthworm, *Pheretima posthuma*. Ten groups were made, each containing six adult earthworms of approximately equal size. The solutions of alcoholic extract, aqueous extract and albendazole were made in the concentrations of 20, 40, 60 mg/ml in normal saline as vehicle. Groups of earthworms were released into 10 ml of desired formulations as made above, and one group was treating as control in normal saline. The observation was made for the time taken to cause paralysis and death of individual worms. Paralysis was said to occur when the worms did not move even in normal saline (Figure1). Death was concluded when the worms lost their motility followed with fading away of their body colours⁷ (Figure2).

Statistical analysis

Data collected in the study are expressed as the 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 Dunnet's test. P value of less than 0.05 was considered to be statistically significant.

RESULTS

Both ethanollic and aqueous extracts of *Butea monosperma* (L) has shown significant effect on *Pheretima posthuma* in concentration dependent manner. Ethanollic extract 40mg/ml has shown significant effect by taking less time to paralyze as well as death ($P<0.05$), similarly concentration of 60mg/ml has shown significant effect on time of paralysis 12 ± 0.683 ($P<0.01$) and time of death 13 ± 0.577 ($P<0.05$) when compared with standard group.

DISCUSSION

The earthworm *Pheretima posthuma* is one of the most important soil invertebrates in promoting soil fertility. These earthworms are important components of the diets of many higher animals⁸.

Helminthic infections of the gastrointestinal tract of human beings and animals have been recognized to have adverse effects on health standards with a consequent lowering of resistance to other diseases. In search of compounds with anthelmintic activity, a number of substances were screened using different species of worms, for example, earthworms, *Ascaris*, *Nippostrongylus*, and *Heterakis*. Of all these species, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro* because they resemble intestinal "worms" in their reaction to anthelmintics and are easily available. It has been demonstrated that all anthelmintics are toxic to earthworms and a substance toxic to earthworms is worthy for investigation as an anthelmintic⁹.

CONCLUSION

In conclusion ethanolic and aqueous extract of *Butea monosperma* (L) bark was assessed for anthelmintic study. The paralysis and death of test organisms justifies the continued use of these plants in folk and traditional medical practice. Studies should therefore be done in order to identify the active phytochemical constituents and evaluate their effectiveness *in vitro* so that they can be synthesized and commercial production begins in earnest.

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Table 1: Phytochemical screening of *Butea monosperma* leaves extracts

Constituents	Ethanolic Extract	Aqueous Extract
Alkaloids	+	+
Sterols	-	-
Glycosides	-	-
Carbohydrates	+	+
Proteins	-	-
Cardiac Glycosides	-	-
Saponins	-	-
Tannins & Phenolic Compounds	+	+
Starch	+	+
Flavonoids	+	+

Table 2: Effect of *Butea monosperma* bark ethanolic and aqueous extract on *Pheretima posthuma* (Earth worm)

Treatment groups	Concentration in mg/ml	<i>Pheretima posthuma</i>	
		Time of paralysis (min)	Time of death (min)
Standard	20 mg/ml	12.5 ± 0.9916	14.83 ± 0.945
	40 mg/ml	11 ± 0.6831	12.17 ± 0.600
	60 mg/ml	6.5 ± 1.176	8.333 ± 0.988
Ethanolic extract	20 mg/ml	13.67 ± 1.585	15.67 ± 1.333
	40 mg/ml	16.50 ± 2.012*	17.5 ± 1.784 *
	60 mg/ml	9.33 ± 1.358	10.83 ± 1.424
Aqueous extract	20 mg/ml	17.67 ± 1.82	18.67 ± 1.961
	40 mg/ml	12.00 ± 0.7303	12.83 ± 0.833
	60 mg/ml	12.00 ± 0.683**	13.00 ± 0.577*
Control	----	----	----

Values are expressed as mean + SEM. and analyzed by One way Analysis of variance (ANOVA) followed by Dunnet's t test n=8, *P< 0.05, **P<0.01, ***P<0.001.

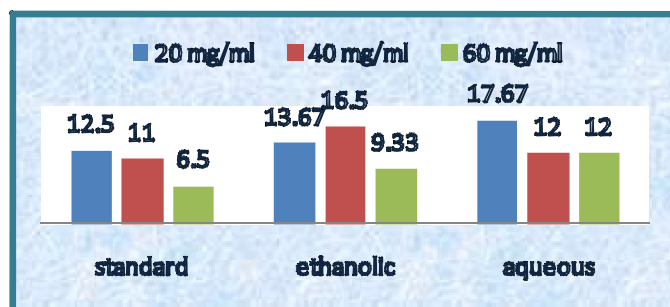


Figure 1: Anthelmintic activity of *Butea monosperma* (L) paralysis time

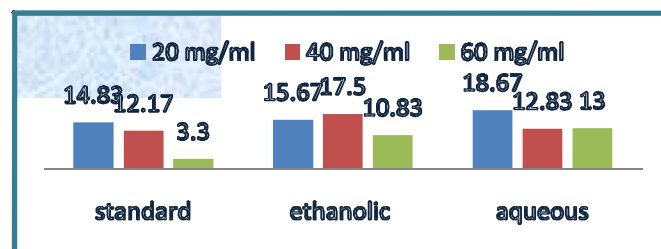


Figure 2: Anthelmintic activity of *Butea monosperma* (L) death time
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