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Evaluation of *in vitro* antihelmintic activity of leaves of *Butea monosperma*

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Abstract

The preliminary phytochemical screening was carried out on the crude extracts of the leaves of *Butea Monosperma* Lam along with ash values and extractive values on the powdered drug. The crude extracts were investigated for their anthelmintic activity against earthworms (*Pheretima posthuma)*, roundworms (*Ascardia galli.)* and tapeworms (*Raillietina spiralis*). Three concentrations (25, 50 and 100 mg/mL) of each extract were studied in activity, which involved the determination of time of paralysis and time of death of the worm. Alcohol and ethyl acetate extracts exhibited significant anthelmintic activity at highest concentration of 100 mg/mL. Albendazole in same concentration as those of extracts was included as standard reference and Di-methyl formamide as control. The anthelmintic activity of alcohol and ethyl acetate extracts of leaves of *Butea Monosperma* has therefore been demonstrated for the first time.

Keywords: Anthelmintic Activity, *Butea Monosperma*, *Pheretima Posthuma. Ascardia Galli*, *Raillietina spiralis*, DMF (di-methyl formamide)

Introduction

Butea monosperma Lam is a wild crop and grows in most parts of India as a tree. It is reputed in systems of medicines as the various parts of the plant *Butea monosperma* has been used traditionally for many of the diseases like anti-inflammatory, antimicrobial, anthelmintic, antidiabetic, diuretic, analgesic, antitumor, anticancer, astringent[1] etc. The leaves and seeds are useful as, in hemorrhage, astringent, diuretic and have anti-implantation and anti-ovulatory properties [2]. Flowers have aphrodisiac and tonic properties. Bark are used in tumors, bleeding piles, ulcers and have inhibitory action against *E.coli* and *Micrococcus pyrogens*. Roots are used to cure night blindness. Chemical component of *Butea monosperma* are alkaloids and recently reported Euphane triterpenoid ester and pterocarpan.Seed contains palasonin,-d-methyl cantharidin, α-amyrin, β-sitosterol and alkaloid- monospermine. Glycerides of palmitic, stearic, linoceric, oleic and linoleic acids, proteolytic and lipolytic enzymes. While bark contains tannins and gum (Butea gum), leucocyanidin and its tetramer procyanidin, gallic acid and mucilaginous material. Its flowers contain isobutin, coreoopsin, monospermoside and their isoderivatives sulphurein, palastrin [3]. Literature survey revealed that the leaf extract has yet not been screened for its traditional claim of anthelmintic activity and hence an attempt has been made in the present study to investigate an anthelmintic activity of crude extracts of leaves of *Butea monosperma*.

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Table 1: *In vitro* Anthelmintic activity of leaves of *Butea monosperma*

Extract	Concentration mg/mL	*Pheretima posthuma*		*Ascardia galli*		*Raillietina spiralis*	
		P	D	P	D	P	D
	25	105	195	108	198	102	192
PE	**50**	75	180	78	182	74	178
	100	48	160	51	162	47	158
	25	101	189	105	197	100	185
HE	**50**	71	170	74	174	70	168
	100	46	155	48	158	47	156
	25	98	181	102	186	96	180
$CHCl_3$	**50**	70	168	73	172	69	164
	100	47	150	49	156	45	152
	25	96	176	101	181	94	172
EtoAC	**50**	68	155	72	158	64	152
	100	45	142	47	144	43	143
	25	95	143	98	146	92	140
EtOH	**50**	68	134	74	138	66	132
	100	44	112	43	114	41	110
	25	55	77	57	79	54	79
AZ	**50**	48	68	49	69	47	67
	100	40	55	41	57	39	43
Control	**-**	-	-	-	-	-	-

PE: Petroleum ether extract, HE: Hexane extract, $CHCl_3$: Chloroform extract, EtoAC: Ethyl Acetate extract, EtOH: Ethanol extract, AZ: Albendazole, P: Time taken for Paralysis (min), D: Time taken for Death of worms (min)

Materials and methods

Extraction of Plant Material

Fresh green leaves of Butea monosperma were collected during the month of July from Arvind herbal labs, Rajapalyam. Tamil Nadu. They were identified by characters mentioned in the literature of various florias [4] and were authenticated by Dr. Stephan, Lecturer, Dept. of Botany, The American College of pharmacy, Madurai. The leaves were washed, powdered and screened through sieve (#40). The powdered drug was then extracted [5] with ethanol for 72 hrs at 60-65 ^{0}C. The ethanol extract was filtered and concentrated to a semisolid mass by vacuum distillation. The green residue was fractioned successively by using petroleum ether, hexane, chloroform, ethyl acetate and ethanol and were used for and phytochemical studies. The residues obtained were formulated as 25, 50 and 100 mg/mL solutions in DMF and investigated for their anthelmintic activity. The powdered drug was utilized for determination of ash values and extractive values.

Table 2: Phytochemical investigation on ethanolic extract fractions of leaves of *Butea monosperma*

Phytoconstituents	Petroleum ether	Hexane	Chloroform	Ethyl acetate	Ethanol Residue
Alkaloids	-	+	+	-	-
Carbohydrates	-	-	-	+	+
Tannins and phenolic	-	-	+	-	-
glycosides	-	-	-	+	+
Flavones	+	-	+	-	-
Phytosterols	+	+	+	-	-

+ Present, - Absent

Physicochemical parameters

Percentage of total ash, water soluble ash, acid insoluble ash and loss on drying were calculated as per Indian pharmacopoeia. Extracts of the powdered leaves were prepared with different solvents for the study of extractive values. The total ash of the powdered leaves was tested for different inorganic constituents [6]. Fluorescence analysis of the powdered leaves was carried out by standard methods [7, 8].

Table 3: Consistency and fluorescence analysis of various extracts of leaves of Butea *monosperma*

Extracts	Consistency	Day light	UV light
Petroleum ether	Sticky mass	Dark green	Green fluorescence
Hexane	Semisolid	Dark green	Green fluorescence
Chloroform	Resinous	Brown green	Brown fluorescence
Ethyl Acetate	Semisolid	Dark green	Green fluorescence
Ethanol Residue	Solid	Brown	Brown fluorescence

Preliminary Phytochemical analysis

For the preliminary phytochemical analysis the ethanolic extract was fractioned successively by using petroleum ether, hexane, chloroform, ethyl acetate and ethanol successively and was used for study. The presence or absence of different phytoconstituents viz alkaloids, carbohydrates, glycoside, tannins flavonoids and phytosterols were detected by usual prescribed methods [9].

Table 4: Ash values of leaves of *Butea monosperma*

Physical constants	Ash value (%) w/w			
	I	II	III	Average (%)w/w
Total Ash	11.50	11.57	11.59	11.55
Acid Insoluble ash	1.26	1.25	1.30	1.27
Water soluble ash	4.56	4.58	4.60	4.58
Loss on drying	8.35	8.40	8.44	8.39

Investigation of anthelmintic activity

All the extracts petroleum ether, hexane, chloroform, ethyl acetate and ethanol of the leaves of *Butea Monosperma* were investigated for their anthelmintic activity against *Pheretima*

posthuma, Ascardia galli and *Raillietina spiralis*. Various concentrations (25, 50 and 100 mg/mL) of each extract were tested in the bioassay, which involved determination of time of paralysis and time of death of the worms. Albendazole was included as standard reference and DMF as control. The anthelmintic assay was carried as per the method of Ajaiyeoba et al [10] with minor modifications. The anthelmintic activity was evaluated on the adults of Indian earth warm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro* [11-13]. Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all faecal matter were used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol. Use of *Ascaridia galli* and *Raillietina* species as a suitable model for screening of anthelmintic drug was advocated earlier [14, 15]. In the first set of experiment, six groups of six earthworms were released in to 50 ml of solutions of Albendazole, petroleum ether, hexane, chloroform, ethyl acetate and ethanol extracts of the leaves of *Butea Monosperma* (25, 50 and 100 mg/mL) in DMF. Albendazole was used as reference standard while DMF as control [16, 17] same experiments were done for *Ascardia galli* and *Raillietina* species. Observations were made on the basis of the time taken for paralysis and/or death of warms. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50 ^{0}C). Treatment with normal saline served as control. Three replications of each treatment were maintained to estimate any sources of error. Paralysis is said to occur when they do not revive even in saline water. Potency is inversely proportional to time taken for paralysis and/or death of warms.

Table 5: Extractive values of leaves of *Butea monosperma*

Solvents	%Extractive value w/w			Average (%) w/w
	I	II	III	
Alcohol Soluble Extractive	9.8	10.1	8.9	9.6
Water Soluble Extractive	15	15.5	14.8	15.1

Results and discussion

The predominant effect of albendazole on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Albendazole by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis. Table 1 show the time (min) taken for the paralysis (P) and death (D) of worms, the results are average of three replications. The standard Albendazole solution has showed enhanced anthelmintic activity. Among the extracts tested, the ethanol and ethyl acetate extracts were the most potent, followed by the chloroform, hexane and petroleum ether extracts. Phytochemical analysis of the crude extracts revealed the presence of flavonoids and phenolic compounds as chemical constituent. Phenolic compounds show anthelmintic activity [18].Some synthetic phenolic anthelmintics e.g. niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation [19]. It is possible that phenolic contents in the extracts of *Butea monosperma* produced similar effects.

Conclusion

In conclusion, the traditional use of leaves of *Butea monosperma* as an anthelmintic have been confirmed as the leaves extracts displayed activity against the worms used in the study when compared to standard Albendazole. Further

studies need to establish the mechanisms (S) of action are required.

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