ANTHELMINTIC POTENTIAL OF CRUDE EXTRACTS AND ITS VARIOUS FRACTIONS OF DIFFERENT PARTS OF *PTEROSPERMUM ACERIFOLIUM* LINN.

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ABSTRACT

The aim of the present study was to determine the anthelmintic activity of crude extracts and different fractions from leaves, barks and flowers of *Pterospermum Acerifolium* Linn. Anthelmintic activity of crude extracts and fractions were investigated against earthworms (*Pheretima posthuma*), roundworms (*Ascardia galli*) and tapeworms (*Raillietina spiralis*) using Albendazole and Piperazine citrate as reference standards. The results of anthelmintic activity revealed that the ethyl acetate fraction of all the parts were most potent which were well comparable with both standard drugs followed by n-butanol fractions of those parts, but at higher doses. All other frations, petroleum extracts and remaining crude extract after fractionations of those three parts of the plant were endowed with minute anthelmintic property, which were not up to standards. The present study prooves the potential usefulness of *Pterospermum Acerifolium* as good anthelmintic agent.

Keywords: *Pterospermum Acerifolium* Linn., Anthelmintic activity, *Pheretima posthuma, Raillietina spiralis, Ascardia galli.*

INTRODUCTION

Resistance of the parasites to existing drugs[1] and their high cost warrants the search for newer anthelmintic molecules. The origin of many effective drugs is found in the traditional medicine practices and in view of this several researchers have undertaken studies to evaluate folklore medicinal plants for their proclaimed anthelmintic efficacy[2]. *Pterospermum acerifolium* (L) Willd (Family: Sterculiaceae) commonly known as "Dinner plate tree" (English) and "Muchukunda" (Hindi), is widely distributed in North Canada and in many parts India i.e. Himalayan tracts, Dehradun, Bengal, Assam and Manipur[3,4]. The flowers are sharply bitter, laxative, disinfectant, anthelminthic, removes cough (In Ayurveda), useful in leucorrhoea, ulcer, inflammation and leprosy. Leaves are used as haemostatic agent[5]. Barks are used as anthelmintic in treating animals[6]. Flavonoids like keampferol, keampferide, luteolin, steroids and triterpenoids like sitosterol, taraxerol, friedelin, sugars, fatty acids are present in the plant[7,8]. As the people consume this plant to cure helminthic infections as per the literature, we attempted to investigate this medicinal plant for its claimed anthelmintic activity.

MATERIALS AND METHODS

Plant material

The plant was identified and authenticated by taxonomist Dr. S. K. Dash- H.O.D of Bioscience; College of Pharm. Science-Mohuda. The voucher herbarium specimen (no.-CPS/HS-008) was diposited in the herbarium of P.G.Dept. of Phytochemistry- College of pharm. Sciences-Mohuda for future reference. After authentication, fresh leaves, barks and flowers were collected separately (during its flowering time in Mar-April-2008) in bulk from young matured plants from the rural hill area of Mohuda, Berhempur - Orissa.

Extraction and fractionation

All the plant materials were washed, shade dried and then milled in to coarse powder by a mechanical grinder. All the powders of those different parts were passed through sieve number 40 and used for further studies. Powder of Leaves (2 kg), barks (2 kg) and flowers (2 kg) were separately extracted successively with petroleum ether (60-80°C) and methanol using Soxhlet apparatus. The solvents were then removed under reduced pressure to obtained sticky residues. The different crude methanolic extracts (leaves-105 g, barks-78g and flowers-89g), after removal of the solvents, were dissolved in 10% sulfuric acid solution and partitioned with chloroform, ethyl acetate and n-buatnol successively to give chloroform fraction (leaf-2.5 g, bark-1.9g,flower-2.2g), Ethyl acetate fraction (leaf-5.5 g, bark-3.9g,flower-4.2g), n-BuOH fraction (leaf-7.8 g, bark-5.9g, flower-6.2g) and remaining crude water soluble fractions (scheme 1).

All extracts, fractions and remaining crude extracts after fractionations were encoded as:

PEL -Pet. Ether Extract of leaf ; CFL-chloroform fraction of leaf ; EFL-ethyl acetate fraction of leaf ; NFL -n-butanol fraction of leaf ; CRL-remaining crude extract after fractionation from leaf ; PEB-Pet. Ether Extract of bark ; CFB-chloroform fraction of bark ; EFB-ethyl acetate fraction of bark ; NFB -n-butanol fraction of bark ; CRB-remaining crude extract after fractionation from bark; PEF-Pet. Ether Extract of flower ; CFF-chloroform fraction of flower ; EFF-ethyl acetate fraction of flower ; NFF-n-butanol fraction of flower; CRF-remaining crude extract after fractionation from flower.

Chemicals & Drugs used- Petrolium Ether (60-80^{0}c), chloroform, ethyl acetate, n-butanol and methanol-(all solvents from Merck Ltd.); saline water (Claris Lifesciences Ltd., Ahmedabad), Albendazole (Alkem Ltd.) and Piperazine citrate (Glaxo Smithkline Ltd.) were used as reference standards for anthelmintic study.

Preliminary phytochemical screening

Standard methods[9, 10] were used for preliminary phytochemical screening of the extracts to know the nature of phytoconstituents present in it (Table. 1).

Anthelmintic activity

Adult earthworms *Pheretima posthuma* L.Vaill (Annelida), Roundworm *Ascaridia galli* Schrank (Nematode) and Tapeworms (*Raillietina spiralis)* were used to evaluate anthelmintic activity in *vitro*. Earthworms were collected near the swampy water, roundworms and tapeworms were obtained from intestine of freshly slaughtered fowls *Gallus gallus* Spadiceus (Phasianidae). Infested intestines of fowls were collected from the local slaughter house and washed with normal saline solution to remove all the faecal matter.

Scheme-1: Scheme of extraction of different parts of P. acerifolium

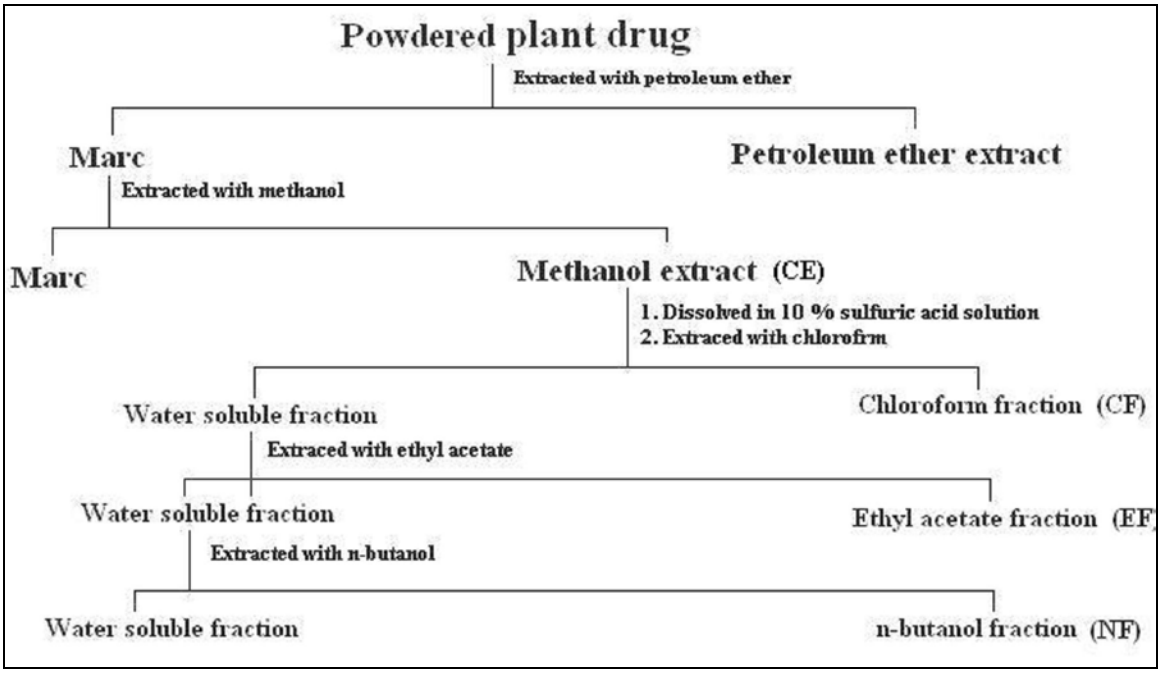

Table 1: Preliminary phytochemical screening of different parts of P. acerifolium

TEST FOR	PEL	CFL	EFL	NFL	CRL	PEB	CFB	EFB	NFB	CRB	PEF	CFF	EFF	NFF	CRF
Alkaloids	+	+	-	-	-	+	-	-	-	-	+	+	-	-	-
Carbohydrates	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+
Glycosides	-	-	+	+	-	-	-	-	+	+	-	-	-	+	+
Triterpenoids	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-
Tannins-phenolic compounds	-	-	+	+	-	-	-	+	+	-	-	+	+	-	-
Protein & amino acids	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Gum & mucilage	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+
Flavones & flavonoids	-	+	+	-	-	-	-	+	+	-	-	+	+	-	-
Saponins	-	-	-	+	+	-	-	-	+	-	-	-	-	+	+
Steroids & sterols	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-

\+ stands for present and – stands for absent

PEL -Pet. Ether Extract of leaf ; CFL-chloroform fraction of leaf ; EFL-ethyl acetate fraction of leaf ; NFL -n-butanol fraction of leaf ; CRL-remaining crude extract after fractionation from leaf ; PEB-Pet. Ether Extract of bark ; CFB-chloroform fraction of bark ; EFB-ethyl acetate fraction of bark ; NFB -n-butanol fraction of bark ; CRB-remaining crude extract after fractionation from bark ; PEF-Pet. Ether Extract of flower ; CFF-chloroform fraction of flower ; EFF-ethyl acetate fraction of flower ; NFF-n-butanol fraction of flower; CRF-remaining crude extract after fractionation from flower.

Table 2: Anthelmintic Activity of leaves of Pterospermum acerifolium
Time taken for paralysis (P) and death (D) of worms in min

	Conc^{n}. Mg/ml	*Pheretima posthuma*		*Ascardia galli*		*Raillietina spiralis*	
		P	D	P	D	P	D
Control	-	-	-	-	-	-	-
PEL	10	59±0.17	92±0.13	58±0.02	--	63±0.54	--
	20	44±0.68	83±0.02	45±0.33	93±0.31	49±0.12	93±0.92
	40	35±0.92	77±0.81	37±0.61	82±0.02	38±0.45	79±0.61
CFL	10	35±0.71	80±0.75	41±0.13	91±0.81	60±0.17	--
	20	27±0.44	66±0.08	32±0.67	79±0.32	45±0.09	87±0.33
	40	21±0.29	59±0.55	24±0.15	69±0.12	33±0.87	74±0.28
EFL	**10**	**23±0.17**	**62±0.73**	**13±0.23**	**65±0.98**	**26±0.32**	**67±0.12**
	20	**17±0.31**	**55±0.12**	**08±0.36**	**53±0.27**	**18±0.66**	**55±0.51**
	40	**13±0.88**	**45±0.77**	**04±0.76**	**42±0.54**	**13±0.43**	**51±0.72**
NFL	10	29±0.12	68±0.54	23±0.23	71±0.13	31±0.64	74±0.79
	20	22±0.07	58±0.11	17±0.61	58±0.11	22±0.93	59±0.09
	40	17±0.28	47±0.76	13±0.32	48±0.65	16±0.65	47±0.02
CRL	10	91±0.11	--	89±0.87	--	78±0.19	97±0.26
	20	83±0.53	--	76±0.47	85±0.88	63±0.56	93±0.61
	40	65±0.67	98±0.86	53±0.12	58±0.23	41±0.33	78±0.38

P=paralysis; D= death, Each value represents mean ± SEM (N=6).

These intestines were then dissected and worms were collected and kept in normal saline solution. The average size of earthworm was 8-9 cm, average size of round worm was 5-7 cm and average size of tapeworm was 6-8 cm. Earthworm and helminths were identified in Dept. of Zoology, Khallikote Aut. College- Berhempur, Orissa and services of veterinary practioners were utilized to confirm the identity of worms.

The anthelmintic assay was carried out as per the method of Ajaiyeoba et al[11]. The assay was performed in *vitro* using adult earthworm (*Pheretima posthuma)* owing to its anatomical and physiological resemblance with the intestinal roundworm parasites *Ascaris lumbricoids* of human beings for preliminary evaluation anthelmintic activity[12,13]. Use of *Ascaridia galli* and *Raillietina* species as a suitable model for screening of anthelmintic drug was advocated earlier[14,15]. Test samples of each extract and fraction was prepared at the concentrations, 10, 20 and 40 mg/ml in distilled water and six worms i.e. *Pheretima posthuma, Ascaridia galli* and *Raillietina spiralis* of approximately equal size (same type) were placed in each nine cm Petri dish containing 25 ml of above test solution of extracts. Albendazole (10 mg/ml) and Piperazine citrate (10 mg/ml) was used as reference standard and saline water as control[16,17,18]. This procedure was adopted for all three different types of worms. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. 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). All the results were shown in (Table. 2, 3, 4) and expressed as a mean ± SEM of six worms in each group.

Table 3: Anthelmintic Activity of barks of Pterospermum acerifolium
Time taken for paralysis (P) and death (D) of worms in min

	Conc^{n}. Mg/ml	*Pheretima posthuma*		*Ascardia galli*		*Raillietina spiralis*	
		P	D	P	D	P	D
Control	-	-	-	-	-	-	-
PEB	10	86±0.12	--	67±0.28	98±0.13	77±0.31	98±0.18
	20	51±0.37	86±0.71	53±0.23	81±0.08	58±0.22	83±0.04
	40	43±0.23	71±0.09	39±0.56	77±0.57	43±0.36	67±0.18
CFB	10	57±0.32	88±0.88	36±0.33	67±0.87	48±0.11	76±0.94
	20	39±0.84	67±0.47	21±0.45	58±0.92	33±0.61	51±0.51
	40	29±0.39	63±0.89	14±0.76	49±0.43	21±0.22	34±0.76
EFB	**10**	**25±0.55**	**64±0.18**	**12±0.22**	**41±0.13**	**24±0.65**	**56±0.38**
	20	**17±0.88**	**48±0.14**	**07±0.12**	**37±0.24**	**17±0.98**	**43±0.87**
	40	**10±0.32**	**43±0.22**	**04±0.56**	**34±0.78**	**12±0.76**	**27±0.62**
NFB	10	31±0.47	67±0.04	15±0.26	43±0.58	25±0.23	61±0.75
	20	20±0.12	53±0.14	11±0.08	33±0.61	19±0.92	47±0.84
	40	12±0.49	47±0.35	07±0.87	15±0.72	13±0.12	30±0.78
CRB	10	89±0.21	--	88±0.61	--	81±0.61	--
	20	71±0.53	--	61±0.28	87±0.22	62±0.54	98±0.75
	40	57±0.67	85±0.86	43±0.12	68±0.23	47±0.33	83±0.38

P=paralysis; D= death, Each value represents mean ± SEM (N=6).

Table 4: Anthelmintic Activity of flowers of Pterospermum acerifolium
Time taken for paralysis (P) and death (D) of worms in min

	Conc^n Mg/ml	*Pheretima posthuma*		*Ascardia galli*		*Raillietina spiralis*	
		P	**D**	**P**	**D**	**P**	**D**
Control	-	-	-	-	-	-	-
PEF	10	76±0.91	--	67±0.13	--	71±0.83	--
	20	63±0.02	95±0.81	48±0.25	78±0.09	59±0.22	91±0.19
	40	49±0.09	83±0.17	33±0.17	68±0.28	40±0.37	74±0.04
CFF	10	61±0.43	92±0.91	49±0.19	81±0.37	59±0.14	92±0.13
	20	45±0.71	79±0.22	31±0.31	64±0.26	41±0.61	74±0.77
	40	29±0.35	64±0.18	19±0.47	49±0.13	29±0.87	61±0.36
EFF	**10**	**24±0.26**	**59±0.19**	**10±0.72**	**37±0.65**	**24±0.27**	**58±0.92**
	20	**19±0.13**	**52±0.24**	**08±0.41**	**31±0.14**	**17±0.81**	**53±0.44**
	40	**13±0.31**	**44±0.37**	**06±0.67**	**24±0.06**	**13±0.17**	**48±0.33**
NFF	10	33±0.31	68±0.22	18±0.12	51±0.59	37±0.19	67±0.27
	20	27±0.61	61±0.07	16±0.29	48±0.51	32±0.17	63±0.91
	40	24±0.04	56±0.61	12±0.23	43±0.42	26±0.31	58±0.11
CRF	10	89±0.49	--	75±0.22	--	80±0.08	--
	20	71±0.53	99±0.17	51±0.66	84±0.54	59±0.88	93±0.76
	40	57±0.67	88±0.86	43±0.12	78±0.23	41±0.33	78±0.38
Albendazole	**10**	**22 ±0.28**	**59± 0.78**	**10±0.14**	**38±0.84**	**22±0.64**	**54±0.26**
Piperazine citrate	**10**	**26±0.68**	**68±0.28**	**15±0.18**	**43±0.78**	**27±0.48**	**58±0.54**

P=paralysis; D= death, Each value represents mean ± SEM (N=6).

RESULTS AND DISCUSSION

In this present study it was observed that the ethyl acetate fractions of all the parts (i.e. EFL, EFB and EFF) were more potent which is well comparable with both standard drugs followed by n-butanol fractions (NFL, NFB and NFF), but at higher doses. Other fractions (CFL, CFB and CFF), petroleum extracts (PEL, PEB and PEF) and remaining crude extract after fractionations (CRL, CRB and CRF) of all the three parts of the plant were endowed with minute anthelmintic property, which were not up to standards.

The order of activity of all the extracts and fractions were:-

EFL>NFL>CFL>PEL>CRL

EFB>NFB>CFB>PEB>CRB

EFF>NFF>CFF>PEF>CRF

The activity revealed concentration dependence nature of the different extracts. Potency of the extracts was found to be inversely proportional to the time taken for paralysis/death of the worms (Table. 2,3,4). Ethyl acetate extracts of all parts of the plant `(showing most potent anthelmintic activity) contains mainly flavones and flavonoids, triterpenoids, phenolic compounds and glycosides may be responsible for the anthelmintic activity[19,20,21,22]. Moderate anthelmintic activity[19,20,21,22,23] of the n-butanolic fractions of different parts may be due to the presence of glycosides, phenolic compounds, saponins and flavonoids present in it. Our results from the present study indicate the potential usefulness of *Pterospermum acerifolium*in the treatment of helminthiasis. Attempts for the isolation and characterisation of the active constituents responsible for such activities are currently under progress. Further studies are necessary to understand the exact mechanism of action.

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