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Research Article

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PHYTOCHEMICAL AND ANTHELMINTIC SCREENING OF CRUDE BARK EXTRACT OF *Adenanthera pavonina* Linn.

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ABSTRACT

Adenanthera pavonina Linn (Family: Leguminosae) is a deciduous fast growing, unarmed tree, found naturally in India. Traditionally it had been used to treat many diseases. The paper presents the physicochemical and anthelmintic studies of bark of *Adenanthera pavonina* Linn. The present investigation has been undertaken with an objective to establish physico- chemical parameters standards, HPTLC profile and *invitro* anthelmintic activity for *Adenanthera pavonina* Linn. bark so that authentic plant material could be explored properly for its traditional claims. The present study will provide the information in respect of its identification and its activity on against *Pheretima posthuma* and *Ascardia galli.*

Keywords: *Adenanthera pavonina* Linn. Bark, Physico-chemical, Fluorescence, phytochemical, HPTLC, *Pheretima posthuma* and *Ascardia galli.*

INTRODUCTION

Adenanthera pavonina Linn (Family: Leguminoceae) is a deciduous fast growing, unarmed tree, found naturally in India . In India it is found in Sub – Himalayan tract, ascending up to an attitude of 1,200 meters in Sikkim, West Bengal, Assam, Meghalaya, Gujarat, Maharashtra, South India & in the Andamans.[1] Traditionally it had been used to treat many diseases**.** Bark and leaves are astringent, vulnerary, anthelmintic and aphrodisiac and are used in colonorrhea, ulcers, pharyngopathy, vitiated conditions of vata and gout and rheumatism.[2] The seeds are bitter, astringent, sweet, cooling, aphrodisiac, antiemetic and febrifuge. They are useful in gout, burning sensation, hyperdipsia, vomiting, fever and giddiness. Powder of the seed is applied as a poultice to abscess to promote suppuration.The heart wood is astrigent, aphrodisiac, heamostatic and is useful in dysentery, haemorrhages and vitiated condition of vata. The roots are reported to be emetic in nature.[3] The present investigation has been undertaken with an objective to establish physicoparameters standards and HPTLC profile and *invitro* anthelmintic activity for *Adenanthera pavonina* Linn. bark so that authentic plant material could be explored properly for its traditional claims.

MATERIALS AND METHODS

The fresh bark of *Adenanthera pavonina* Linn. were collected in the month of September (2007) from salipur, Orissa, India. These were identified, confirmed and authenticated by Prof. P. Jayaraman, PARC, Chennai.

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The voucher specimen was given the No. PARC/2007/82. Collected fresh bark were washed and used to evaluate physiochemical parameters. The powder of dried bark was used for the determination ash values, extractive values and phytochemical investigations. Among the different extracts, ethanolic extract was used to carry out its HPTLC profile and to evaluate its anthelmintic activity. All chemicals and reagents used for testing were analytical grade obtained from SD Fine Chemicals, Mumbai (India).

Extraction

The powdered material was extracted successively with petroleum ether (60-80°), ethyl acetate, chloroform and methanol by using soxhlet apparatus. The solvent was removed under reduced pressure which gave light yellow, green, deep green, dark brown, colored residue for petroleum ether, ethyl acetate, chloroform, and ethalonic extract respectively. The extracts were concentrated under vacuum at 40-60°c which yields a residue (3.2 w/w, 4.7w/w, 10.24%w/w 16.78w/w,) which were stored in a desiccators at room temperature.[4]

Physico-chemical parameters

Percentage of total ash, acid-insoluble ash, water soluble ash and sulphated ash were calculated as per the Indian Pharmacopoeia.[5] The total ash of the powdered bark was tested for different inorganic elements. Different extracts of the bark were prepared for the study of extractive values.[6] Fluorescence analysis of powdered bark was carried out by standard methods.[7, 8]

Preliminary phytochemical analysis

For the preliminary phytochemical analysis, 5 g powdered drug was extracted with petroleum ether (60-80), ethyl acetate, chloroform and ethanol successively. The extracts were dried and weighed. The presence or absence of

different phytoconstituents viz. triterpenoids, steroids, alkaloids, sugars, tannins, glycosides and flavonoids, etc. were detected by usual prescribed methods.[9]

High performance thin layer chromatography

Based on chemical test and thin layer chromatography of various extracts of bark of the plant that ethanolic bark extract was found to have more number of phytoconstituents. So further attempt was taken to separate the individual components of ethanolic bark extract by HPTLC instrument, CAMAG Linomat 5 taking Chloroform: Methanol: Formic acid (8.5:0.5:1) as solvent system.

In vitro Anthelmintic activity

Ethanolic extracts from the bark of *Adenanthera pavonina* Linn. were investigated for their anthelmintic activity against *Pheretima posthuma* and *Ascardia galli*. Various concentrations (25, 50 and 100 mg/ml) of ethanolic extract were tested in the bioassay, which involved determination of time of paralysis and time of death of the worms. Piperazine citrate was included as standard reference and distilled water as control. The anthelmintic assay was carried as per the method of Ajaiyeoba et al., 2001 with minor modifications.[10]

In the first set of experiment, three groups of six earthworms i.e. *Pheretima posthuma* were released in to 50 ml of solutions of piperazine citrate, and ethanolic extracts of bark of *Adenanthera pavonina* linn. (25, 50 and 100 mg/ml each) in distilled water. Observations were made for the time taken to paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colors. Same experiment was done for *Ascardia galli* worms only the difference was solutions were prepared in normal saline solutions.

RESULTS

Behavior of bark powder of *Adenanthera pavonina* Linn. with different chemical reagents were performed to detect the occurrence of phytoconstituents along with color changes under ordinary daylight by standard method which is tabulated in Table**1**

Table1. Behavior of bark powder with different chemical reagents

S No.	Acid/ reagent	Observation
1	powder as such	light brown
2	Powder + picric acid	yellow
3	Powder + conc. Nitric acid	red
4	Powder + con. Hydrochloric acid	light green
4	Powder + conc. Sulphuric acid	brown
6	Powder + Glacial acetic acid	colorless
7	Powder + 5% Ferric chloride solution (aqueous)	green
8	Powder + Sodium hydroxide(5N)	yellow
9	Powder + Potassium hydroxide (5%)	light yellow
10	Powder + Iodine/20	red

Physico-chemical study

The percentage of total ash, acid-insoluble ash, water soluble ash, sulphated ash and different extractives are tabulated in Table **2** and **3**. The qualitative analysis of ash indicated presence of calcium, aluminum, potassium, chlorides and sulphates.

Table 2. Ash Value *Adenanthera pavonina* Linn.bark

Type of ash	%w/w (Mean±SEM)
Total ash	7.55±0.167
Acid insoluble ash	1.76±0.062
water soluble ash	4.41±0.071
Sulphated ash	6.45±0.256

Mean value of six readings

Table 3. Extractive values of *Adenanthera pavonina* Linn. bark with different solvents

Types of solvent	% Extractability (Mean±SEM)
Petroleum ether	0.24±0.05
Benzene	0.60±0.02
Ethyl acetate	1.26±0.07
Chloroform	1.63±0.04
Methanol	6.28±0.07
Water	2.53±0.05

Mean value of six readings

Fluorescence characteristics

When physical and chemical parameters are inadequate as it often happens with the powdered drugs, the plant material may be identified from their adulterants on basis of fluorescence study of different extract and powdered drug which is tabulated in Tables **4** and **5.**

Table 4 Fluorescence analysis of different solvent extract of *Adenanthera pavonina* Linn. bark under UV and visible light

Extract	Visible light	Ultra Violet	
		Short wave	Long wave
Petroleum extract (60-80°C)	light yellow	yellowish green	greenish black
Chloroform extract	yellowish green	green	greenish brown
Ethyl acetate extract	light brown	light green	black
Methanol extract	light brown	green	dark brown

Table 5. Fluorescence analysis of bark powder of *Adenanthera pavonina* Linn. with different chemical reagents.

Reagent	Color in day light	Short wave UV	Long wave UV
Powder as such	Light brown	Light brown	Dark brown
Powder+1 N NaOH in Methanol	Light green	Green	Yellowish green
Powder+1 N NaOH	Yellowish green	Green	Brown
Powder+ Ethanol	Colorless	Colorless	Colorless
Powder+ HNO_3+NH_3 sol.	Light green	Light green	Brown
Powder+50% HNO_3	Light green	Green	Brown
Powder + 1 N HCl	Colorless	Colorless	Light brown
Powder+HCl	Light green	Light green	Brown
Powder+H_2SO_4	Deep brown	Black	Black
Powder+50% H_2SO_4	Light green	Green	Brown
Powder+glacial acid	Colorless	Colorless	Light brown
Powder+HNO_3	Light yellow	Light green	blue

Preliminary phytochemical analysis

The preliminary phytochemical analysis of bark extracts of petroleum ether (60-80°C), ethyl acetate, chloroform and ethanol are tabulated in Table **6**.

High performance thin layer chromatography

HPTLC profile showed the separation of 6 different phytoconstituents having different retention factor. The

results of HPTLC of ethanolic bark extract are shown in form of chromatogram **1**, plate **1** and Table **7**.

Table 6. Qualitative phytochemical analysis of various extracts of *Adenanthera pavonina* Linn. bark

Types of constituent	Petroleum ether	Ethyl acetate	Chloroform	Ethanol
Alkaloid	-	-	-	+
Carbohydrate and glycoside	-	-	+	+
Saponin	-	+	-	+
Protein	-	-	-	-
Sterol	+	+	+	+
Fixed oils and fats	+	-	+	-
Phenolics and flavonoids	-	-	-	+
Gums and mucilage	-	-	-	-

Chromatogram 1. HPTLC of ethanolic bark extract

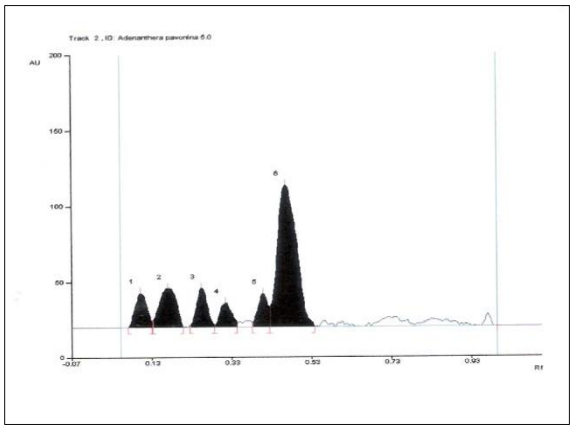

Plate 1. HPTLC of ethanolic bark extract

Table 7. HPTLC of Ethanolic Bark Extract

Peak	Start Rf	Start height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %
1	0.07	0	0.11	22.8	11.06	0.14	3.9	516.1	8.43
2	0.14	4	0.17	25.8	12.52	0.21	0.1	850.7	13.9
3	0.23	1.6	0.26	25.7	12.48	0.29	1.6	530	8.66
4	0.29	1.7	0.32	16.1	7.8	0.35	2.8	360	5.88
5	0.39	3.1	0.41	22.2	10.75	0.43	13.3	423.8	6.92
6	0.43	13.5	0.47	93.6	45.4	0.54	0	3440	56.2

Solvent system- Chloroform : Methanol: Formic acid (8.5:0.5:1) HPTLC: High Performance Thin Layer Chromatography

In vitro Anthelmintic activity

Preliminary phytochemical screening of ethanolic extract revealed the presence of anthraquinone glycosides, phenolic compounds and steroids. From the results shown in **Table 8**, the predominant effect of piperazine citrate on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis.

Table 8. Athelmintic activity of ethanolic extracts of *Adenanthera pavonina* Linn.

Treatment	concentration mg/ml	Pheretima posthuma		Ascardia galli	
Ethanolic extract		P	D	P	D
	25	63.73±0.85	70.2±0.45	61.04±0.95	78.5±0.45
	50	42±0.22	64±0.12	45.5±0.15	67.2±0.1
	100	22±0.95	32±0.45	32.2±0.6	44.7±0.23
Piperazine citrate	25	1.6±0.82	53±0.4	40.5±0.15	53.5±0.45
	50	0.95±0.11	29.5±0.12	28±0.5	30.4±0.1
	100	0.55±0.17	19.5±0.80	21.5±0.3	23±0.85
Control(distilled water)	-	-	-	-	-

Where, P: Time taken for Paralysis of worms (min) D: Time taken for Death of worms (min)

The ethanolic extract of *Adenanthera pavonina* Linn. demonstrated paralysis as well as death of worms in a comparable time as compared to piperazine citrate especially at higher concentration of 100 mg/ml.

DISCUSSION

The water soluble ash is almost half of total ash and twice of acid insoluble ash. The alcohol soluble extractive value is more than any other extractive value indicating the solubility of phytoconstituents in alcohol. The fluorescence analysis of powder and extract indicate the any fluorescent phytoconstituent present or adulterants. Preliminary phytochemical analysis indicates the nature of phytoconstituents present in different solvent extract. This also indicates that the ethanol extract have more number of phytoconstituent than any other extracts i.e. Carbohydrates, alkaloids, glycosides, phytosterol, saponin flavonoids and phenolics. These are few of the important physico-chemical characters of the bark. HPTLC profile showed the separation of six different phytoconstituents

having different retention factor which may be the phytochemicals we got in preliminary phytochemical analysis of ethanolic bark extract. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis. Phytochemical analysis of the crude extracts revealed presence of flavonoids as one of the chemical constituent. Polyphenolic compounds show anthelmintic activity.[11] It is possible that phenolic content in the extracts of *Adenanthera pavonina* Linn. might have interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation which might have paralyzed and eventually resulted the death of both species of the worm.[12]

CONCLUSION

It can be concluded from this study that bark extracts of *Adenanthera pavonina* Linn. possess significant anthelmintic activity. In the current research anthelmintic activity of the bark of the plant was explored next to its traditional claims.

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