

Original Article

## Phytochemical evaluation, anthelmintic and analgesic activities of butanol fraction of *Adenanthera pavonina L.* bark extract

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### ABSTRACT

*Adenanthera pavonina L.* (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 phytochemical evaluation, anthelmintic and analgesic studies of butanol fraction of *Adenanthera pavonina L.* The ethanol bark extract was fractionated with pet ether, chloroform ethyl acetate, butanol and concentrated under vacuum at 40-60°C which yields a residue (3.3 w/w, 4.8w/w, 6.26%w/w 7.78w/w) which were stored in a desiccators at room temperature. It can be concluded from this study that butanol fraction of ethanol bark extract of *Adenanthera pavonina L.* possess significant anthelmintic and analgesic activity.

## 1. INTRODUCTION

*Adenanthera pavonina L.* 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 Andaman's [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 astringent, aphrodisiac, hemostatic and is useful in dysentery, hemorrhages 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 phytochemical investigation of different fractions of ethanol extract of bark and to establish the *in-vitro* anthelmintic and analgesic activity for butanol fraction *Adenanthera pavonina L.* bark.

## 2. EXPERIMENTAL

The fresh barks of *Adenanthera pavonina L.* were collected from Salipur, Orissa, India. These were identified, confirmed and authenticated by Prof. P. Jayaraman, PARC, Chennai. The voucher specimen was given the No. PARC/2007/82. Collected fresh barks were washed and used for phytochemical investigations of different solvent fractions ethanol extract. All chemicals and reagents used for testing were analytical grade obtained from SD Fine Chemicals, Mumbai (India).

### 2.1 Extraction

The powdered material was extracted successively with petroleum ether (60-80°), ethyl acetate, chloroform and ethanol 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 ethanol extract respectively. The ethanol extract was further fractionated with pet ether, chloroform ethyl acetate, butanol and concentrated under vacuum at 40-60°C which yields a residue (3.3 w/w, 4.8w/w, 6.26%w/w 7.78w/w,) which were stored in a desiccators at room temperature [4-8].

## 2.2 Preliminary phytochemical analysis

For the preliminary phytochemical analysis, pet ether, chloroform, ethyl acetate, butanol fractions 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].

## 2.3 In-vitro anthelmintic activity

*Adenanthera pavonina L.* bark butanol fraction was investigated for their anthelmintic activity against *Pheretima posthuma* and *Ascaridia galli*. Various concentrations (25, 50 and 100 mg/ml) of butanol fraction 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 with minor modifications [10, 11].

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 butanol fraction of *Adenanthera pavonina L.* (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 *Ascaridia galli* worms only the difference was solutions were prepared in normal saline solutions.

## 2.4 Evaluation of analgesic activity by acetic acid induced writhing model

The peripheral antinociceptive action of *Adenanthera pavonina L.* butanol fraction of bark extract bark was carried out using acetic acid induced abdominal writhing reflex pain model. Eighteen mature mice were randomly divided into 3 groups (n=6) fasted for 12 hours. Group I served as control, Group II of rats was administered with butanol fraction (200 mg/kg, p.o.) and Group III was administered with acetylsalicylic acid (50 mg/kg, p.o.). The control group received with vehicle (0.9% normal saline in 1% tween 80 (2 ml) using stomach tube. 0.6% glacial acetic acid (10 ml/kg) was administered intraperitoneally (I.P) to all the mice 30 minutes after the administration of extracts and reference drug for inducing abdominal constriction or writhing's. The analgesic effect was assessed in each mouse for 30 minutes and recorded [12].

The percentage of inhibition is calculated as:

$$\% \text{ of inhibition} = \frac{\% \text{ of inhibition}}{\text{Control}} \times 100$$

## 3. RESULTS AND DISCUSSION

### 3.1 Preliminary phytochemical analysis

The preliminary phytochemical analysis of bark extracts of petroleum ether (60-80°C), ethyl acetate, chloroform and butanol fraction of ethanol extract of bark are tabulated in Table 1.

**Table 1.** Qualitative phytochemical analysis of various fraction of *Adenanthera pavonina L.* bark

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

**Table 2.** Anthelmintic activity of butanol fraction of *Adenanthera pavonina L.* bark

Treatment	Concentration mg/ml	<i>Pheretima posthuma</i>		<i>Ascaridia galli</i>	
		P	D	P	D
Butanol fraction	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)

### 3.2 In-vitro anthelmintic activity

Preliminary phytochemical screening of butanol fraction revealed the presence of anthraquinone glycosides, phenolic compounds and steroids. From the results shown in Table 2, the predominant effect of piperazine citrate on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. The butanol fraction of *Adenanthera pavonina L.* 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.

### 3.3 Acetic acid induced writhing model

The analgesic effect results of *Adenanthera pavonina L.* (200 mg/kg) on acetic acid induced writhing reflex method is presented (Table 3). The results showed that the butanol fraction 200 mg/kg and reference drug acetyl salicylic acid significantly reduced the mean number of abdominal constrictions or writhing. As compared to the control group  $79.5 \pm 2.377$ , the butanol fraction of bark extract and acetyl salicylic acid reduced the mean number of abdominal constrictions or writhing significantly up to  $27.66 \pm 1.406$  (65.20%) and  $20.83 \pm 2.120$  (73.79%) ( $P < 0.01$ ) respectively. In this model, butanol fraction of 200 mg/kg showed significant inhibition of abdominal constrictions or writhing formation when compared to vehicle treated control group.

**Table 3.** Analgesic activity of butanol fraction of *Adenanthera pavonina L.* bark

Groups	Treat-ment group	Dose	No. of writhing in 30 minutes $\pm$ SEM	% Inhibition
Groups I	Control	2 ml vehicle	$79.5 \pm 2.377$	---
Groups II	Butanol fraction	200 mg/kg	$27.66 \pm 1.406^{**}$	65.20
Groups III	Aspirin	50 mg/kg	$20.83 \pm 2.120^{**}$	73.79

Data are the mean  $\pm$  SEM values for six rats in each group using the ANOVA followed by Dunnet's t test. \* $p < 0.05$ , \*\* $p < 0.01$  as compared to the control.

Preliminary phytochemical analysis indicates the nature of phytoconstituents present in different fraction of ethanol extract. This also indicates that the butanol fraction have more number of phytoconstituent than any other extracts i.e. Carbohydrates, alkaloids, glycosides, phytosterol, saponin flavonoids and phenolic. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyper polarization 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. It is possible that phenolic content in the butanol fraction of *Adenanthera pavonina L.* might have interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation. With respect to the acetic acid-induced abdominal writhing which is the peripheral pain model the result has shown that butanol fraction produced significant analgesic effect. Abdominal constriction induced by acetic acid is used to screen peripheral analgesic effect mediated by local peritoneal receptor. Effect of the butanol fraction of bark ethanol

extract on acetic acid induced abdominal constriction suggested that they might inhibit or modify responses to pain mediated by nociceptors peripherally.

### 4. CONCLUSION

It can be concluded from this study that butanol fraction of ethanol bark extract of *Adenanthera pavonina L.* possess significant anthelmintic and analgesic activity. After the above screening procedure it is confirmed that the butanol fraction of ethanol extract of *Adenanthera pavonina L.* bark were found to contain effective therapeutic principles which may be held responsible for exhibiting the therapeutic activity such as anthelmintic and analgesic activities which was also confirmed from its pharmacological screening using different animal models. Further study can be extended to establish the plant stable dosage form to exploit it commercially to be utilized as alternative remedies to the available synthetic therapeutic agents.

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