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Analgesic, Antifungal and *In-Vitro* Cell Cytotoxic Studies of *Bauhinia Variegata* Bark Extract

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ABSTRACT

Bauhinia variegata (Mountain ebony) is a well known plant in traditional medicine which belongs to the family Cesalpiniaceae. It is widely used for various ailments like skin diseases, tumour, ulcers and various infections. Against this backdrop the present study attempts to scientifically evaluate the analgesic, antifungal, anti-tumour potential of this plant. The ethanolic extract of the plant was prepared by soxhlation. Acute toxicity studies carried out on the extract revealed that it was safe at the dose of 2000 mg/kg body weight. The extract was then evaluated for its analgesic activity by eddy's hot plate method. The antifungal evaluation was carried against C*andida albicans* and *Aspergillus fumigatus* by disc diffusion method in Sabouraud agar medium. The extract was studied for short term *in-vitro* cytotoxicity against DLA cell lines by Trypan blue assay method. The ethanolic extract of *Bauhinia variegata* bark exhibited significant analgesic activity at both the doses of 200 and 400 mg/kg body weight ($p<0.05$). It possesses *in-vitro* cell cytotoxicity against DLA cell lines (IC_{50} 153). It also showed good antifungal activity against *Aspergillus fumigatus* and *Candida albicans*. The MIC was found to be 4.15 µg/ml against both organisms.

Keywords: *Bauhinia variegata*, analgesic, antifungal, Trypan blue assay.

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INTRODUCTION

In India, plant is one of the major sources of drug used by the traditional healers and in ayurvedic system of medicine. Even though India has one of the richest biodiversities in the world, there is no exclusive and reliable scientific information available about many of these plants used by different ethnic communities. There is a need for continuous search for novel compounds in the plant kingdom with minimal side effects which will be beneficial for a sizable chunk of population.

The plant *Bauhinia variegata* is known as Kanchanara in Hindi and used for a number of ailments in traditional Ayurvedic medicine[1].The plant is distributed in sub Himalayan tracts and in forests of India[2] .The bark of the plant is used to treat skin diseases, ulcers and also against tumours[2]. The present investigation evaluated the ethanolic bark extract of *Bauhinia variegata* for analgesic, antifungal and anti-tumour activity.

MATERIALS AND METHODS

Collection of plant material and extraction

The bark of the plant *Bauhinia variegata* was obtained from Kulai, Mangalore. It was authenticated by Neoline J. Pinto, H.O.D, Dept. of Botany, St. Agnes College, Mangalore. The dried bark was coarse powdered in a cutter and grinding mill. The powdered bark is then subjected to extraction with ethanol in a soxhlet apparatus[3]. The extract was concentrated with the help of a vacuum evaporator and kept in a desiccator.

Preliminary phytochemical screening and animals used

The bark extract prepared were then subjected to qualitative tests for the identification of various plant constituents such as alkaloids, glycosides, phytosterols, saponins, amino acids and carbohydrates[4]. Albino rats were used for carrying out acute toxicity studies by OECD guidelines 425, analgesic activity was done by using albino mice and the protocol was approved by the institutional animal ethics committee (KSHEMA/AEC/14/2010).

Analgesic activity studies[5]

Analgesic activity of the bark extract was carried out by Eddy's hot plate method. Albino mice of either sex weighed between 25-30 g were selected for study. In this experiment, hot plate was maintained at 55 ± 5℃. A cut off period of 15 sec was observed to avoid damage to the paw. Reaction time was recorded when animals licked their fore or hind paws or jump response which ever appeared first. The basal reaction time was noted before and 30, 60, 90 and 120 min after the administration of the drugs. The animals were divided into four groups of six animals in each group. Group I served as control (received 1% tween 80, 5 ml/kg i.p) Group II served as standard

and given pentazocaine (5 mg/kg i.p) body weight. The groups III and IV received the test drugs 200 and 400 mg/kg body weight respectively.

Antifungal studies

The antifungal potential of the extract was studied against *Candida albicans* (ATCC no 2091) and *Aspergillus fumigatus* (ATCC no 36607) in Sabouraud's agar medium using disc diffusion method. Fluconazole was used as the standard drug. The minimum inhibitory concentration studies were carried out in BHI broth using tube dilution method.

***In-vitro* cytotoxicity studies[6]**

The *Bauhinia variegata* bark extract was evaluated for *in-vitro* cell cytotoxic activity against Dalton's Lymphoma Ascites cells (DAC) by trypan blue assay method. DAC cells (1×10^6) were incubated with the bark extract at various concentrations at 10, 20, 50, 100, 200, 500 µg/ml in phosphate buffered saline at 37˚C for 3 h. Viable cells were counted in a hemocytometer .

RESULTS AND DISCUSSION

Non- opioid analgesics act by inhibiting prostaglandin synthesis[7]. The main adverse effects associated with these drugs are gastric ulceration and renal damage. Mycosis is referred as the infection caused by fungi. There are a large number of topical and systemic agents available to treat these infections which are often life threatening. In the last two decades candidema is more common because of an increasing number of immunocompromised patients.

In this study we tried to evaluate the analgesic, antifungal and anti-tumour potential of plant *Bauhinia variegata* bark as indicated in the literature survey. The bark extract was prepared by soxhlation using ethanol as a solvent. Acute toxicity studies carried out on this extract as per OECD guidelines 425 showed that it was safe at the dose level of 2000 mg/kg body weight. $1/10^{th}$ and $1/20^{th}$ of this maximum safe dose (200 and 400 mg/kg) was taken for further pharmacological evaluation. The bark extract was found to possess significant analgesic activity at both the dose levels of 200 and 400 mg/kg body weight. The results were comparable with that of standard drug pentazocaine ($p<0.05$). Data is tabulated in Table-1.The extract showed promising antifungal activity against the yeast *C. albicans* and the mold *A. fumigatus*. The zone of inhibition obtained at various concentrations of the extract against these organisms was comparable with standard flucanazole (Table-2). The minimum inhibitory concentration (MIC) was recorded at 4.15 (µg/ml) against both the organisms (Table-3). In the preliminary screening the extract showed significant cell cytotoxicity against DLA cell lines by trypan blue assay method (IC_{50} 153). Results are shown in Table-3.

Table 1: Analgesic activity of *Bauhinia variegata* bark extract by Eddy's hot plate method

Groups	Treatment n=6	Reaction time (second) 0 min	30 min	60 min	90 min	120 min
I	Control (Tween-80)	2.40±0.09	2.36 ± 0.16	2.23 ± 0.15	2.38 ± 0.07	2.20 ± 0.04
II	Pentazocine[a] (5 mg/kg, i.p)	2.30±0.04	5.61±0.53**	8.45±0.24**	7.96±0.04**	7.73±0.15**
III	BBEE (200 mg/kg)	2.11±0.06	3.16±0.15**	3.78±0.37**	4.21±0.33**	4.23±0.64**
IV	BBEE (400 mg/kg)	2.50±0.16	3.96±0.36**	4.66±0.22**	5.21±0.18**	5.95±0.27**

ANOVA followed by Dunnet's multiple comparison test used. All values are expressed as mean ± SEM; **p< 0.01; BBEE-*Bauhinia variegata* bark ethanolic extract; [a]standard

Table 2: Antifungal activity of *Bauhinia variegata* bark extract (zone of inhibition)

Zone of inhibition in mm				
Microorganism	**BBEE (µg/ml)**			**Fluconazole**
	75	**50**	**25**	**25**
C. albicans	22.67±0.33	18.33±0.33	13.67±0.66	20±0.88
A. fumigatus	24.33±0.33	21.67±0.33	18.33±0.33	23.67±0.88

BBEE-*Bauhinia variegata* bark ethanolic extract. Values are average reading of three independent determinations ± SEM

Table 3: Antifungal activity of *Bauhinia variegata* bark extract (MIC)

Test drug	Minimum inhibitory concentration (µg/ml)	
	C. albicans	***A. fumigatus***
Fluconazole	16.6	8.3
BBEE	4.15	4.15

BBEE-*Bauhinia variegata* bark ethanolic extract

Table 4: Cell cytotoxic activity studies of *Bauhinia variegata* bark extract against DLA cell lines

Test drug	Concentration(µg/ml)	DLA(% Cytotoxicity)	IC_{50}
Curcumin[a]	10	5.0 ± 0.00	88 µg/ml
	20	9.6±0.33	
	50	39.17±0.40	
	100	52.67±0.49	
	200	72.0±0.57	
	500	100±0.00	
	Control	0.0±0.00	
BBEE	10	5.63 ± 1.32	153 µg/ml
	20	5.66 ± 0.84	
	50	21.50 ±1.47	
	100	22.17± 1.77	
	200	40.83 ± 1.66	
	500	63 ± 0.68	
	Control	0.00±0.00	

[a]standard; BBEE- *Bauhinia variegata* bark ethanolic extract

CONCLUSION

Our studies concluded that ethanolic extract of *Bauhinia variegata* bark possesses significant analgesic, antifungal and anti-tumour activity as mentioned in the traditional medicine. There is a scope for further studies regarding the chemical constituents present in the extract and evaluation of cell cytotoxicity against different cell lines (against normal and tumour) which gave more insight about the therapeutic value of this plant.

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