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IN-VITRO CYTOTOXIC EFFECTS OF METHANOLIC EXTRACTS OF *CAESALPINIA PULCHERRIMA*

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ABSTRACT

The aim of this research was to investigate cytotoxic effects of crude extracts of *Caesalpinia pulcherrima* (Family: Fabaceae). The flower of *Caesalpinia pulcherrima* was extracted with methanol and methanolic extract was fractionated into three fractions like n-Hexane, ethyl acetate and chloroform. The methanolic crude extracts were screened for cytotoxic properties using brine shrimp lethality bioassay. A reputed cytotoxic agent vincristine sulphate was used as a positive control. From the results of the brine shrimp lethality bioassay it can be well predicted that n-hexane, ethyl acetate, chloroform soluble fractions of *Caesalpinia pulcherrima* flower possess cytotoxic principles (LC₅₀ 1.940µg/mL, 2.704µg/mL and 8.359µg/mL respectively) comparison with positive control vincristine sulphate (LC₅₀ 0.563 µg/mL).

Keywords:

Caesalpinia pulcherrima, fabaceae flower, crude extracts cytotoxic effects

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INTRODUCTION: *Caesalpinia pulcherrima* (Local name: Krishnachura or Radhachura, Family: Fabaceae) is an evergreen, low-branching and fast growing shrub that can grow up to 4 m tall. Canopy is round, moderately dense and wide spreading with smooth outline. Occasional pairs of thorns can be seen at nodes. Leaves are bi-pinnately compound and opposite or sub-opposite in arrangement and 20 to 30 cm long.

Each leaf has four to six pairs of pinnae and each pinna has 7 to 15 pairs of leaflets, which are oblong or ovate in shape, 1 to 1.5 cm long and have smooth margin. Inflorescence is a corymb. Flowers are very showy, large, red, orange or yellow in color.

Each flower has five sepals and five petals and the fifth petal is far smaller than the other four. Fruit is a pod, which is flat, compressed, and green when young, brown when ripe; each pod is about 10 cm long and contains five to six seeds¹.

Many medicinal compounds have isolated from *Caesalpinia pulcherrima* like bonducellin⁹, diterpenoid^{3, 21, 26}, diterpene ester⁶, furanoid ditekene^{7, 22, 23}, furanoid²⁴, flavonoid^{18, 28}, homoisoflavone^{8, 14}, polysaccharide¹³ and vauacapen-5α-ol¹⁰.

Caesalpinia pulcherrima has been used as a potent medicinal agent in antimicrobial^{2, 29}, antioxidant⁴, antibacterial⁴, antiviral⁵, larvicidal¹¹, ovidical¹¹, repellent¹¹, anthelmintic¹⁷, antiulcer²⁷, anti-inflammatory²⁵, anti-tubercular²², antioxidant¹⁹, cytotoxic activity¹⁹ and protease inhibitor¹².



METHODS AND MATERIALS:

Collection of the Plant Sample: Plant sample of *Caesalpinia pulcherrima* was collected from Noakhali in April, 2010.

Extraction of the Plant Material: About 800 gm of the powdered material was taken in a clean desiccator and soaked with methanol (2.5 L). The container with its content was sealed by foil and kept for a period of 15 days accompanying occasional shaking and stirring. The whole mixture was then filtered through filter paper and the filtrate thus obtained was concentrated in open air dry.

Preparation of Mother Solution: Methanolic crude extract (5 g) of *Caesalpinia pulcherrima* was triturated with methanol (90 mL) and distilled water (10 mL). The crude extract went to the solution completely and the mother solution was partitioned off successively with three solvents (n-hexane, ethyl acetate and chloroform) of different polarity by Kupchan modified method (**Figure 1**). The amount of extracts was n-hexane (0.18 g), ethyl acetate (0.24 g) and chloroform (0.21 g).

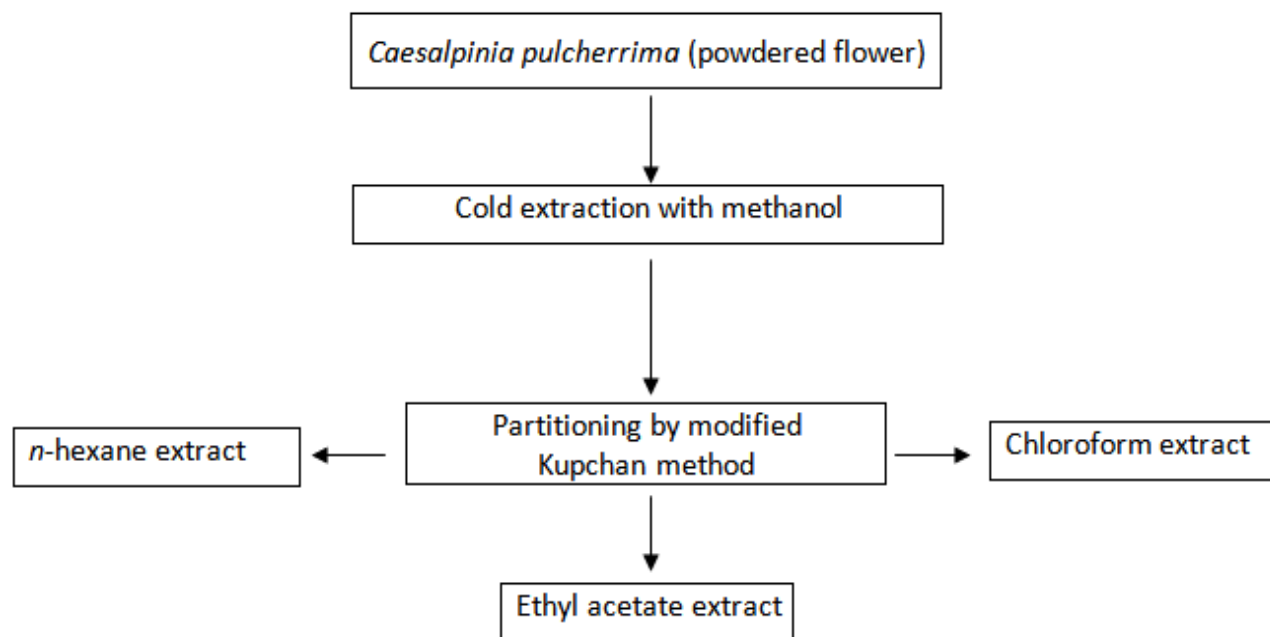


FIGURE 1: SCHEMATIC DIAGRAM OF THE CRUDE EXTRACTS OF CAESALPINIA PULCHERRIMA

RESULT AND DISCUSSION: The Brine Shrimp Test (BST) represents a rapid, inexpensive and simple bioassay for testing plant extract lethality which in most cases correlates reasonably well with cytotoxic and anti-tumour properties¹⁵. Following the procedure of Meyer¹⁶ and Persoone²⁰ the cytotoxic effects of the

methanolic crude extracts, n-hexane, ethyl acetate and chloroform soluble fractions were determined. The LC₅₀ values of n-hexane, ethyl acetate and chloroform soluble fraction found to be 1.940 µg/mL, 2.704 µg/mL and 8.359 µg/mL respectively with a positive control, vincristine sulphate (LC₅₀ 0.563 µg/mL) (**Table 1**).

TABLE 1: LC₅₀ VALUES OF THE METHANOLIC CRUDE EXTRACTS OF CAESALPINIA PULCHERRIMA

Methanolic extracts	LC ₅₀ (µg/mL)	Regression equation	R ²
n-hexane	1.940	Y = 36.18x- 20.22	0.655
Ethyl acetate	2.704	Y = 24.06x-15.07	0.548
Chloroform	8.359	y = 6.470x-4.086	0.486
Vincristine sulphate (positive control)	0.563	y = 30.056x + 56.016	0.9168

TABLE 2: EFFECT OF n-HEXANE, ETHYL ACETATE and CHLOROFORM SOLUBLE FRACTION ON BRINE SHRIMP NAUPLII

Conc. (C) ($\mu\text{g/mL}$)	Log C	Mortality (%)			LC ₅₀ ($\mu\text{g/mL}$)			Vincristine sulfate			
		n-hexane	Ethyl acetate	Chloroform	n-hexane	Ethyl acetate	CF	Conc (C) ($\mu\text{g/mL}$)	Log C	Mortality (%)	LC ₅₀ ($\mu\text{g/mL}$)
400	2.602	100	80	20	1.940	2.704	8.359	40	1.602	100	0.563
200	2.301	100	60	20				20	1.301	90	
100	2	30	10	0				10	1.000	90	
50	1.699	10	0	0				5	0.698	80	
25	1.398	10	0	0				2.5	0.397	70	
12.5	1.097	0	0	0				1.25	0.096	70	
6.25	0.796	0	0	0				0.625	-0.204	50	
3.125	0.495	0	0	0				0.3125	-0.505	30	
1.56	0.193	0	0	0				-	-	-	
0.78	-0.108	0	0	0				-	-	-	

CONCLUSION: The present research indicates that the crude extracts of *Caesalpinia pulcherrima* has got intense *in-vitro* cytotoxic effect and may have potential use in traditional medicine. From the previous studies and our current investigation, it may be concluded that further study can be carried out to investigate the individual bioactive principles.

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