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CYTOTOXIC ACTIVITY OF METHANOLIC EXTRACTS OF *JUSTICA ADHATODA*

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ABSTRACT

The aim of the study is to observe the cytotoxic activitiy of methanolic crude extracts of root of Justica adhatoda a plant belonging to the family Acanthaceae. The root of Justica adhatoda was extracted with organic solvent and the extracts were used for the observation of cytotoxic activitiy. Crude extracts (n-hexane, ethyl acetate and chloroform soluble fraction) of Justica adhatoda, were screened for cytotoxic activity 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 and chloroform soluble fraction of methanolic crude extracts possess cytotoxic principles (with LC_{50} 1.129μg/ml, LC_{50} 1.402 μg/ml and LC_{50} 2.130 μg/ml respectively) comparison with positive control vincristine sulphate (with LC_{50} 0.563 μg/ml).

INTRODUCTION: *Justica adhatoda* (Synonym: Adhatoda vasica , Family: Acanthaceae, Local name: Vasaka) is a dense evergreen shrub between 1.2-2.4 meters high, with long ascending branches covered in a yellowish bark, oppositely arranged. The glabrous leathery leaves are borne on short petioles, elliptic-lanceolate, tip acute, minutely hairy when young. The flowers arise in short, dense terminal pedunculate spikes with large bracts, the corolla white, streaked pink or purple within. Bloom Period: April, May, June. The fruit is a small club-shaped capsule with longitudinal channels, containing 4-6 seeds [1]. Many medicinal compounds had been isolated from Justica adhatoda like podophyllotoxin lignan [2], vasicine and vasicinone [3] and 4, 6-diphenyl-2-pyrimidinylamine [6]. *Justica adhatoda* is being used as a potential medicinal agent in hepatotoxic activity [4], anti-inflammatory [5], anti-tuberculosis [7], antimicrobial activity 8, genotoxicity [9], larvicidal activity [10], modulatory influence [11], protective effect [12], antiplasmodial activity [13], anti-ulcer activity [17], fungio-oxidant activity [18], antioxidant activity [19], red spider mite actvity 20 and anticestodal activity [21].

MATERIALS AND METHODS:

Collection of the plant: Plant sample of Justica adhatoda was collected from Gajipur in February 2010.

Plant Material preparation: The root of the plant was collected in fresh condition. It was sun-dried and then, dried in an oven at reduced temperature (not more than 500C) to make it suitable for grinding purpose. The coarse powder was then stored in air-tight container with marking for identification and kept in cool, dark and dry place for future use.

Solvent-solvent partitioning of Crude Extract: The crude extract is diluted with sufficient amount of aqueous alcohol (90%) and then gently shaken in a separating funnel with almost equal volume of a suitable organic solvent (such as n hexane) which is immiscible with aqueous alcohol.

The mixture is kept undisturbed for several minutes for separation of the organic layer from the aqueous phase. The materials of the crude extract will be partitioned between the two phases depending on their affinity for the respective solvents. The organic layer is separated and this process is carried out thrice for maximum extraction of the samples. After separating of the organic phase, the aqueous phase thus obtained is successively extracted with other organic solvents, usually of the increasing polarity (such as n-hexane, chloroform, ethyl acetate). Finally all the fractions (organic phases as well as the aqueous phase) were collected separately and evaporated to dryness.

Extraction of the plant material: Fresh root of Justica adhatoda was collected, dried and ground to a coarse powder. The powder sample (724 g) was subjected to cold extraction with methanol for about 15 days. The methanol extract was then subjected to modified Kupchan partitioning method with n-hexane, ethyl acetate and chloroform. Thus three extractives like n-hexane (0.04 g), chloroform (4 g) and ethyl acetate (0.04 g) were obtained.

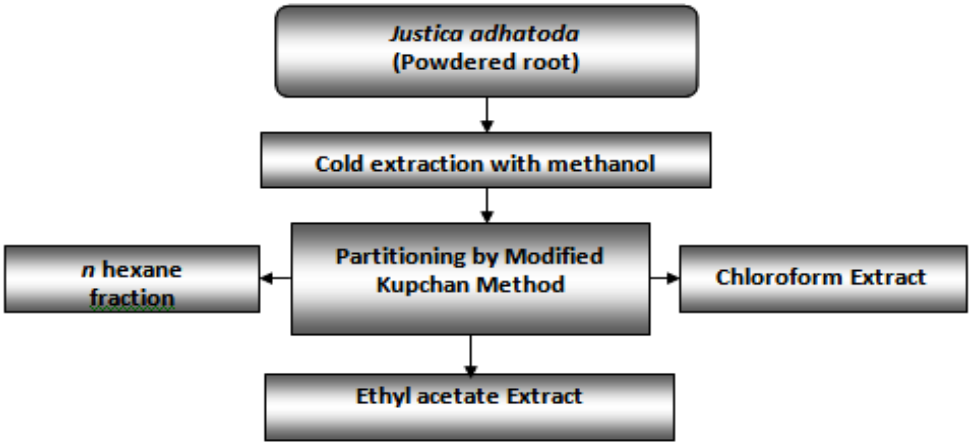

FIG 1: SCHEMATIC DIAGRAM OF THE CRUDE EXTRACT OF *JUSTICA ADHATODA*

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 properties [14]. Following the procedure of Meyer [15, 16], the cytotoxic activity of crude extracts (ethyl acetate, n -hexane and chloroform soluble fractions) are shown in **table 1** and effect of methanolic extract on brine shrimp nauplii are shown in **table 2**. It is observed that the LC_{50} values of n-hexane, ethyl acetate and chloroform soluble fraction found to be 1.129µg/ml 1.402 and 2.130µg/ml respectively. The positive control vincristine sulphate showed LC_{50} at a concentration of 0.563µg/ml. From the results of the brine shrimp lethality bioassay it can be well predicted that the n-hexane, ethyl acetate and chloroform soluble fractions possess cytotoxic principles. Comparison with positive control vincristine sulphate signifies that mild antitumor and pesticidal activity.

TABLE 1: LC_{50} VALUES OF METHANOLIC CRUDE EXTRACTS OF *JUSTICA ADHATODA*

Methanolic crude extract (soluble fractions)	LC_{50} (µg/ml)	Regression equation	R^2
Vincristine sulphate (positive control)	0.563	y = 30.056x + 56.016	0.9168
n-hexane	1.129	y = 33.12x + 12.60	0.965
Ethyl acetate	1.402	y = 32.88x + 3.901	0.950
Chloroform	2.130	y = 21.58x + 4.026	0.937

TABLE- 2: EFFECT OF METHANOLIC EXTRACT (N-HEXANE, ETHYL ACETATE & CHLOROFORM SOLUBLE FRACTION) ON BRINE SHRIMP NAUPLII

Conc. (C) (µg/ml)	Log C	% Mortality			LC_{50} (µg/ml)			Vincristine Sulfate			
		n-hexane	Ethyl acetate	CF	*n*-hexane	Ethyl acetate	CF	Conc. (C) (µg/ml)	Log C	% Mortality	LC_{50} (µg/ml)
400	2.602	90	90	60	1.129	1.402	2.130	40	1.602	100	0.563
200	2.301	90	70	50				20	1.301	90	
100	2	80	70	50				10	1.000	90	
50	1.699	70	60	40				5	0.698	80	
25	1.398	60	60	30				2.5	0.397	70	
12.5	1.097	60	40	30				1.25	0.096	70	
6.25	0.796	40	40	30				0.625	-0.204	50	
3.125	0.495	30	20	50				0.3125	-0.505	30	
1.56	o.217	20	00	00				0.156	-0.81	30	
0.78	-1.08	00	00	00				0.78	-1.11	10	

CONCLUSION: The present study indicates that the crude extracts of *Justica adhatoda* has got intense cytotoxic effect and may have potential use in 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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