



EVALUATION OF IN VITRO CYTOTOXIC ACTIVITY OF DIFFERENT EXTRACTS OF *CRATEAVA MAGNA* LEAF

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ABSTRACT

Crataeva magna (Lour.) DC belonging to family Capparaceae is a high-value medium sized deciduous medicinal tree of tropical climate found in tropical regions of the world and also grows almost all over India, especially in the semiarid regions. The present investigation is designed to conduct phytochemical screening of *Crataeva magna* leaves after each successive extraction with petroleum ether, n-hexane, chloroform, methanol and water followed by its *in vitro* cytotoxic activity study.

Key words: *Crataeva magna*, Soxhlet extraction, Phytochemical screening, *In vitro* cytotoxicity.

INTRODUCTION

India has rich ancient heritage of traditional medicine¹. From last two decades, the utility of medicinal plants have been phenomenally increased due to their vast chemical biodiversity as World Health Organization advocated traditional medicines as safe remedies². The conventional therapeutic experiences of an array of bioactive phytoconstituents from those species, over hundreds years are considered as valuable remedial recipe to treat various acute and chronic disorders. Among them *Crataeva magna* Buch-Ham (Family: Capparidaceae) commonly known as Varuna, is a well explored traditional Indian medicinal plant used to treat various ailments in particular urolithiasis³. It is a medium sized branched deciduous plant distributed throughout the river banks of Westernghat region of southern India, wild or cultivated⁴. Vedic literatures described its potentiality as blood purifier and to maintain homeostasis⁵. Traditionally, the stem bark is used as stomachic, laxative, anthelmintic, expectorant and anti-pyretic⁶. Moreover, pharmacological study reveals the potentiality of *Crataeva magna* extract and its active principle, particularly lupeol as diuretic,

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anti-inflammatory, antioxidant, cardio-protective, hepatoprotective, lithonotriptic, anti-rheumatic, anti-periodic, contraceptive, anti-protozoal, rubifacient and vesicant⁷. Preliminary phytochemical screening reveals the plant is rich in secondary metabolites like alkaloids, saponins, triterpenes, tannins, flavanoid glycosides, glucosinolates and phytosterols⁸. Phytoconstituents like ceryl alcohol, friedelin, cadabicine diacetate, betulinic acid and diosgenin have already been isolated from the stem bark⁹. Since, the plant posses diverse medicinal properties, the present work had been designed to evaluate the in-vitro cytotoxicity of different extracts of leaf of *Crateava magna*.

EXPERIMENTAL

Collection and authentication of plant material

The fresh leaves of the plant *crateava magna* were collected from botanical garden, University of Calicut, Kerala state, India and authetified by Dr. P. S. Udayan, Taxonomist, Sree Krishna College, Kerala and the voucher specimen number was 842 and was deposited in Sree Krishna College, Guruvayur Herbarium, Trissur district of Kerala state. The collected leaves were washed, shade dried and pulverized with mechanical pulverizer for size reduction and passed through #60 and that powder was used for preparation of extract.

Method

Extraction procedure

The powdered plant material was successively extracted by using Soxhlet extractor. 132 g of powdered drugs were placed in a central compartment of Soxhlet assembly. 750 mL of solvent was placed in a lower compartment and a reflux condenser is attached above the central compartment. The vapour passes through the side-arm up into the reflux condenser. Here the vapour liquified and drips into the plant material to be extracted. The warm solvent percolates through the materialband the extracts gradually collects in the central compartment. Once the height of the extract reached the top of the siphon, the entire liquid flowed through this and back to the lower solvent container. The process is then repeated. The extract collected in the lower vessel, gradually becoming more and more concentrated. The vapour rising from the heated extract is pure solvent vapour and so the liquid dripping into the material from the condenser is essentially pure solvent, though derived from the extract. After complete extraction the lower vessel was removed, solvent recovered and the extract is concentrated and percentage yield was calculated. The solvents were recovered by using simple distillation method. The charged drug from the central

compartment was removed, dried, recharged and extracted with N-hexane. By using Soxhlet extractor exhaustive extraction with a series of solvents of increasing polarity was done. Solvents used with increasing polarity: Petroleum ether, Benzene, Chloroform, Methanol and finally water. The percentage yield of extracts produced is given in table no1.

Table 1: Percentage yield of Leaf extracts of *Crateava magna* with different solvents

S. No.	Extracts	% Yield of extract (w/w)
1	Petroleum ether extract	1.341
2	N-Hexane extract	0.947
3	Chloroform extract	2.222
4	Methanolic extract	1.668
5	Aqueous extract	1.606

Evaluation cytotoxicity

The test compounds were studied for short term *in vitro* cytotoxicity using Dalton's lymphoma ascites cells (DLA). The tumour cells aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with PBS or normal saline. Cell viability was determined by trypan blue exclusion method. Viable cell suspension (1×10^6 cells in 0.1 mL) was added to tubes containing various concentrations of test compounds and the volume was made up to 1ml using phosphate buffer saline. Control tube contained only cell suspension. These assay mixture was incubated for 3 hrs at 37°C. further cell suspension was mixed with 0.1 mL of 1% trypan blue and kept for 2-3 min and loaded on a haemocytometer. Dead cells take up the blue colour of trypan blue while live cells do not take up the dye. The number of stained and unstained cells were counted separately.

$$\% \text{ Cytotoxicity} = \frac{\text{No. of dead cells}}{\text{No. of live cells} + \text{No. of dead cells}} \times 100$$

RESULTS AND DISCUSSION

The different Extracts of *Crateava magna* were subjected to *in-vitro* cytotoxic activity by using Dalton's lymphoma ascites cells (DLA). The study was shown significant cytotoxic activity for all the extracts at different concentrations. The percentage of cell death is shown in the Table 2. More percentage of cell death was shown by N-hexane and Chloroform extracts at concentration 200 µg/mL.

Table 2: Percentage cell death

Extracts	Percentage cell death				
	10 µg/mL	20 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL
Petroleum ether extract	6	12	28	40	48
N-Hexane	20	32	44	70	90
Chloroform	14	28	30	58	88
Methanol	0	3	8	14	20
Water	0	0	8	6	12

The different Extracts of *Crateava magna* were subjected to *in-vitro* cytotoxic activity by using Dalton's lymphoma ascites cells (DLA). The study was shown significant cytotoxic activity for all the extracts at different concentrations. However, further investigations using carcinoma cell line are necessary to isolate the active compounds responsible for activity.

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