



IN VITRO CYTOTOXIC EFFECT OF METHANOLIC CRUDE EXTRACTS OF *TERMINALIA CHEBULA*

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ABSTRACT

The aim of this work is to observe *invitro* cytotoxic effect of methanolic crude extracts of *Terminalia chebula* (Family: Combretaceae). The bark of *Terminalia chebula* were soaked with methanol and then methanolic crude extract was fractionated into *n*-hexane, ethyl acetate, and chloroform soluble fractions. The methanolic crude extracts (*n*-hexane, ethyl acetate, and chloroform soluble fractions) of *Terminalia chebula* were screened for cytotoxic activity using brine shrimp lethality bioassay. A reputed cytotoxic agent vincristine sulphate used as a positive control. From the results of the brine shrimp lethality bioassay it can be well predicted that methanolic crude extracts (*n*-hexane, ethyl acetate and chloroform soluble fractions) of *Terminalia chebula* possess cytotoxic principles, (LC₅₀ 1.413 µg/mL, 1.492 µg/mL and 1.496 µg/mL respectively) comparison with positive control, vincristine sulphate (LC₅₀ 0.563 µg/mL).

key words: *Terminalia chebula*, Fabaceae, Combretaceae, methanolic crude extract and cytotoxic effects.

INTRODUCTION

Terminalia chebula (Local name: haritaki, family: Combretaceae) is a tree tall about 50-80 ft in height. It has rounded crown and spreading branches. The bark is dark brown with some longitudinal cracks. Leaves are ovate and elliptical, with two large glands at the top of petiole. The fruit or drupe is about 1-2 inches in size. Fruit is green when unripe and yellowish grey when ripe. Fruits are collected from January to April. Each fruit has a single seed that is light yellow in color. The flowers are dull white with spikes and can be found at the end of the branches¹. *Terminalia chebula* is called the “king of medicines” in Tibet and is always listed first in the Ayurvedic materia medica because of its extraordinary powers of healing. Many medicinal compound have been isolated from *Terminalia chebula* like tannins⁴, chebulagic acid¹² and tannase production¹⁵. *Terminalia chebula* has been used as a potencial medicinal agent in HIV-1 integrase inhibition², radiation protection³, growth inhibition⁵, antioxidant effects^{6,11}, cytoprotective effect⁹, renoprotective¹¹, cancer cell growth inhibitor¹³, liver toxic agents¹⁸ and myocardial infection¹⁷ and in elucidation of antimicrobial¹⁴ & metabolic constituents¹⁶.

METHODS AND MATERIALS

Collection of the Plant

Plant sample of *Terminalia chebula* (bark) was collected from Noakhali, April 2010.

Extraction of Plant Material

The powdered material of *Terminalia chebula* (bark, 700 g) were soaked with methanol (2.0 L) in August 15, 2010 in a desicator with occasional shaking and stirring for 25 days. The extract was then filtered through filter-cloth. The filtrate was kept to dry in fresh and clean air to afford a mass of cytotoxic investigation.

Preparation of mother solution

Methanolic extract of *Terminalia chebula* bark (5 g) was triturated with ethanol (100 mL) containing distilled water (10 mL). The crude extract went to the solution completely. This is the mother solution, which was partitioned off successively by three solvents (*n*-hexane, chloroform and ethyl acetate) of different polarity by kupchan method (Figure-1). The amount of crude extracts were found in *n*-hexane (1.5 g), chloroform (1 g) and ethyl acetate (0.5 g).

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 properties⁷. Following the procedure of Meyer and persoon^{8,10} the cytotoxicity of the methanolic crude extracts (*n*-hexane, ethyl acetate and chloroform fractions) of *Terminalia chebula* were determined. The LC₅₀ values of *n*-hexane, ethyl acetate and chloroform soluble fraction found to be 1.413 mg/mL, 1.492 mg/mL and 1.496 mg/mL respectively. The positive control vincristine sulphate showed LC₅₀ at a concentration of 0.563 mg/mL (Table-1). 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. The cytotoxic effect of methanolic crude extracts on brine shrimp nauplii are given in table-2.

CONCLUSION

The present study indicates that the crude extracts of *Terminalia chebula* have 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.

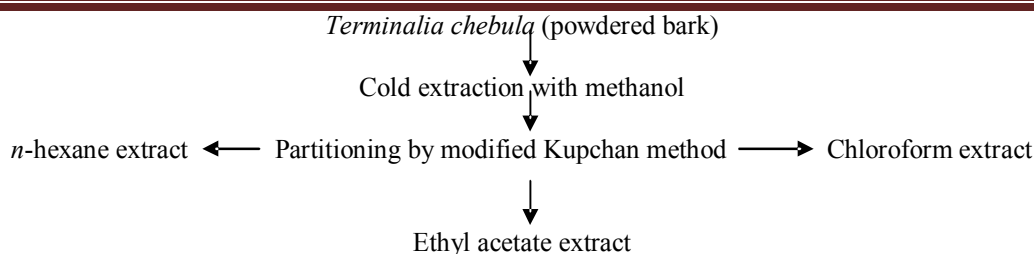


Figure-1: Schematic diagram of the crude extracts of *Terminalia chebula*

Table -1: Cytotoxic effects of crude extracts of *Terminalia chebula*

Sample (soluble fractions)	LC ₅₀ (µg/mL) based on log C	Regression equation	R ²
n-hexane	1.413	y = 48.89x - 19.10	0.780
Ethyl acetate	1.492	y = 45.46x - 17.81	0.848
Chloroform	1.496	y = 44.65x - 16.80	0.847
Vincristine sulphate (positive control)	0.563	y = 30.056x + 56.016	0.9168

Table-2: Cytotoxic effect of methanolic crude extracts on brine shrimp nauplii

Conc (C) (µg/mL)	Log C	% Mortality			LC ₅₀ (µg/mL)			Vincristine Sulfate			
		n-hexane	Ethyl acetate	Chloroform	n-hexane	Ethyl acetate	CF	Conc (C) (µg/mL)	Log C	% Mortality	LC ₅₀ (µg/mL)
400	2.602	100	100	100	1.413	1.492	1.496	40	1.602	100	0.563
200	2.301	100	100	100				20	1.301	90	
100	2	100	100	100				10	1.000	90	
50	1.699	100	40	40				5	0.698	80	
25	1.398	20	40	30				2.5	0.397	70	
12.5	1.097	0	10	10				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				0.156	-0.807	30	
0.78	-0.108	0	0	0				0.078	-1.108	10	

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