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BRINE SHRIMP LETHALITY BIOASSAY OF METHANOLIC CRUDE EXTRACTS OF *TERMINALIA ARJUNA*

Md. Anaet Hossen Maruf¹ and Mohammad Musarraff Hussain *²

Department of Pharmacy, Noakhali Science and Technology University¹, Sonapur, Noakhali-3802, Bangladesh
Department of Pharmacy, Jagannath University², Dhaka-1100, Bangladesh

ABSTRACT

The aim of this work is to determine the cytotoxic activities of *Terminalia arjuna* (Family: Combretaceae) by brine shrimp lethality bioassay. The leaves of *Terminalia arjuna* were extracted with methanol and the methanolic extract was fractionated into three fractions by using organic solvents like n-hexane, ethyl acetate and chloroform. The n-hexane, ethyl acetate and chloroform soluble fractions of methanolic crude extract were screened for antitumor 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 could be well predicted that n-hexane, ethyl acetate and chloroform soluble fractions of *Terminalia arjuna* leaves possess cytotoxic principles (LC₅₀ 1.29 µg/mL, 1.5 µg/mL and 1.23 µg/mL respectively) comparison with positive control, vincristine sulphate (LC₅₀ 0.563 µg/mL).

Keywords:

Terminalia arjuna, Combretaceae, Methanolic extracts, Cytotoxic activities, Brine shrimp lethality bioassay

Correspondence to Author:

Mohammad Musarraff Hussain
Assistant Professor, Department of
Pharmacy, Jagannath University, Dhaka-
1100 Bangladesh

E-mail: m.musarraff.hussain@gmail.com

INTRODUCTION: *Terminalia arjuna* (Local name: Arjun, Family: Combretaceae) is a tree about 60-80 feet in height, large, evergreen with a spreading crown and drooping branches. It is simple, grey and smooth on external surface. The bark is thick, soft and of red color from inside, glabrous and often in equilateral. Leaves are oblong, 4-6 inch long, 2-3 inch wide, sub opposite.

The fruits are 1-1.5 inch in diameter with 5-7 longitudinal lobes, glabrous with five to seven wings, woody, fibrous, drupe, often notched near the top and marked with oblique upward curving striations¹. Lot of medicinal agents and been isolated from *Terminalia arjuna* like olenane triterpine glycoside², ursane triterpine²¹, cytotoxic agents¹⁷ and free radical scavenger²¹.

Terminalia arjuna had been used as a potent medicinal agent in healing activity³, gastro-protective⁴, ulcer protective⁵, cardiovascular disorder⁶, anti-inflammatory⁷, Immunomodulatory⁷, anti-nociceptive

, myocardial fibrosis⁸, inhibitory effects⁹, phytomedicinal activity¹⁰, cardioprotective¹¹, antioxidant¹⁵, adriamycin-induced DNA damage¹⁶, antigenotoxic properties¹⁸ and protective effects¹⁹.

METHODS AND MATERIALS:

Collection and proper identification of the plant sample: Plant sample (Leaves) of *Terminalia arjuna* was collected from Bhola in April, 2010.

Preparation of Mother Solution: Powdered material of the plant (450 g) was taken in a clean desiccator (5 L) and soaked with methanol (1.5 L).



The

container

with its content was sealed by foil and kept for a period of 20 days accompanying occasional shaking and stirring. The whole mixture was then filtered through filter paper and the filtrate thus obtained was concentrated at open air dry.

Extraction of the Plant Material: Methanolic extract of *Terminalia arjuna* leaves (5 g) was triturated with

methanol (100 mL) containing distilled water (10 mL). This is the mother solution, which was partitioned off successively in three solvents (n-hexane, ethyl acetate and chloroform) of different polarity by modified Kupchan partitioning method and thus three extractives like n-hexane (1.1 g), chloroform (0.04 g) and ethyl acetate (0.6 g) were obtained.

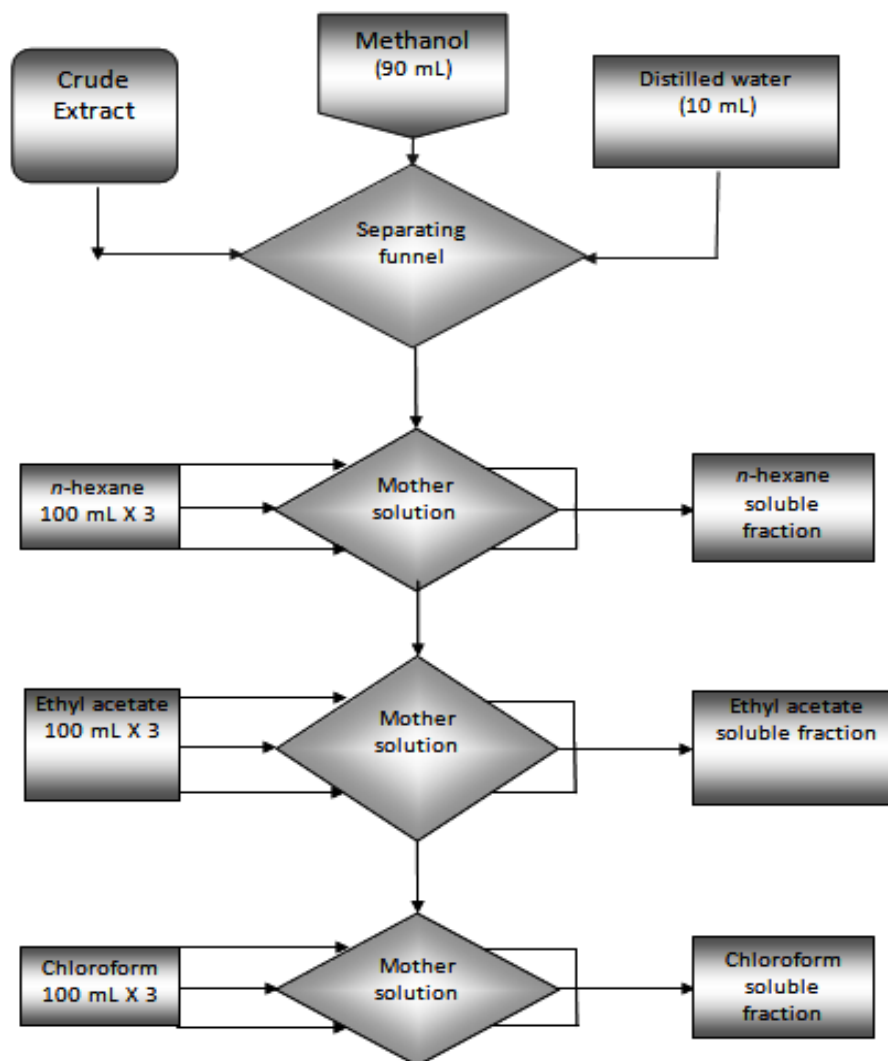


FIGURE 1: SCHEMATIC DIAGRAM OF A MODIFIED KUPCHAN PARTITIONING METHOD

RESULT AND DISCUSSION: The brine shrimp lethality bioassay is a rapid, inexpensive and simple testing method of cytotoxic and anti-tumor properties determination of plant extract¹¹. Following the procedure of Meyer, 12, 14 the cytotoxicity of methanolic crude extracts (n-hexane, ethyl acetate and

chloroform soluble fractions) were determined and the LC₅₀ values of n-hexane, ethyl acetate and chloroform soluble fraction found to be 1.29 mg/mL, 1.5mg/mL and 1.23 mg/mL respectively comparison with the positive control, vincristine sulphate (**Table 1**).

TABLE 1: LC₅₀ VALUES OF METHANOLIC CRUDE EXTRACTS OF *TERMINALIA ARJUNA*

Methanolic crude extracts (soluble fractions)	LC ₅₀ (µg/mL)	Regression equation	R ²
n-hexane	1.29	Y = 45.08x – 8.235	0.918
Ethyl acetate	1.5	Y = 43.67x – 15.47	0.897
Chloroform	1.23	Y = 44.88x – 4.984	0.902
Vincristine sulphate (positive control)	0.563	Y = 30.056x + 56.016	0.9168

TABLE 2: EFFECT OF n-HEXANE, ETHYL ACETATE & CHLOROFORM SOLUBLE FRACTION ON 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	1.29	1.5	1.23	40	1.602	100	0.563
200	2.301	90	100	100				20	1.301	90	
100	2	90	70	100				10	1.000	90	
50	1.699	90	60	90				5	0.698	80	
25	1.398	60	50	60				2.5	0.397	70	
12.5	1.097	40	10	20				1.25	0.096	70	
6.25	0.796	10	0	20				0.625	-0.204	50	
3.125	0.495	0	0	10				0.3125	-0.505	30	
1.56	0.193	0	0	10							
0.78	0.108	0	0	0							

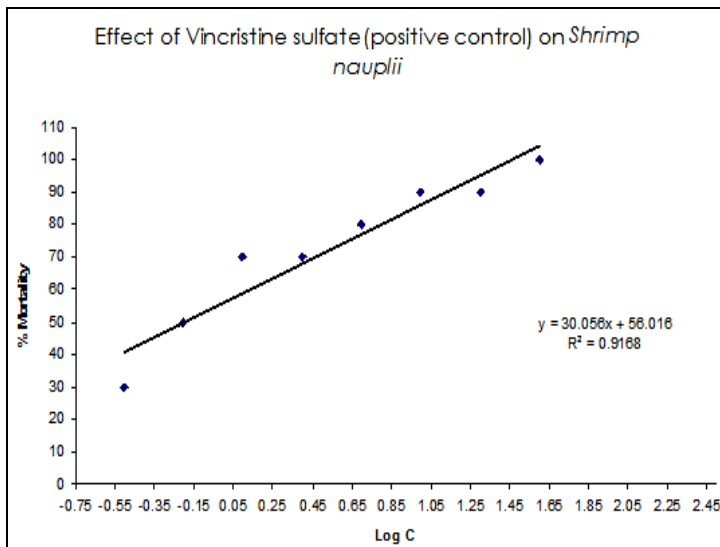


FIGURE 2: EFFECT OF VINCRISTINE SULPHATE ON BRINE SHRIMP NAUPLII DEPENDS ON LOG C

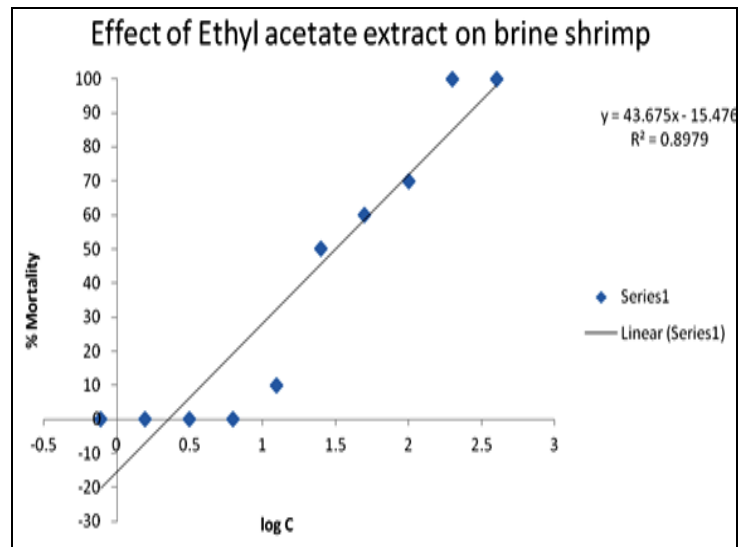


FIGURE 4: EFFECT OF ETHYL ACETATE SOLUBLE FRACTION ON BRINE SHRIMP NAUPLII

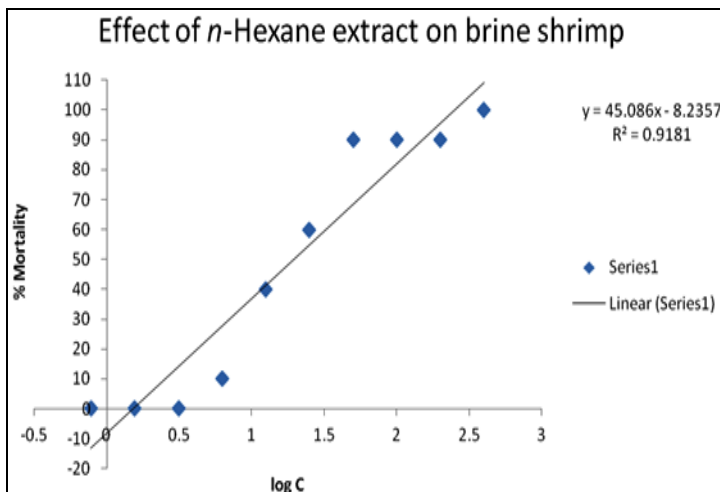


FIGURE 3: EFFECT OF n-HEXANE SOLUBLE FRACTION ON BRINE SHRIMP NAUPLII

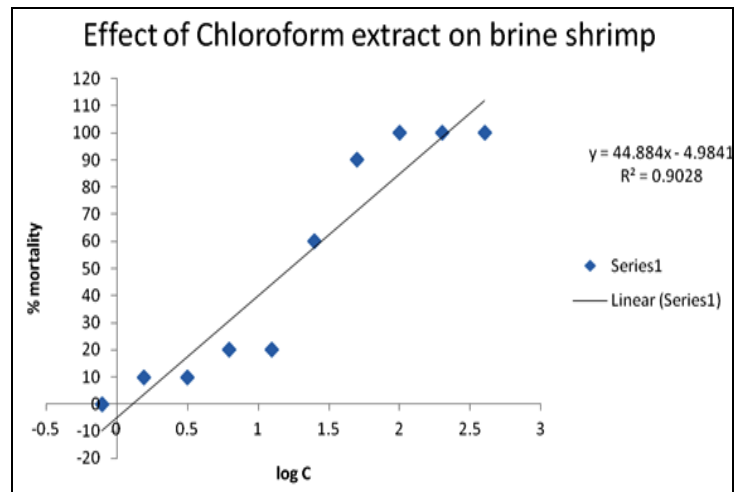


FIGURE 5: EFFECT OF CHLOROFORM SOLUBLE FRACTION ON BRINE SHRIMP NAUPLII

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