



Received on 25 November, 2012; received in revised form, 21 February, 2013; accepted, 29 March, 2013

ASSESSMENT OF CYTOTOXIC EFFECT OF METHANOLIC CRUDE EXTRACTS OF *AZADIRACTA INDICA*

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Keywords:

Azadirachta indica, Meliaceae, Methanolic Crude Extracts, Cytotoxic Activity

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ABSTRACT: The aim of the research is to observe the cytotoxic activity of methanolic crude extracts of *Azadirachta indica*, a plant belonging to the family Meliaceae. The root of *Azadirachta indica* was extracted with organic solvent and the extracts were fractionated by using three fractions (n-hexane, ethyl acetate and chloroform). The crude extracts 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 can be well predicted that n-hexane, ethyl acetate, chloroform soluble fractions of *Azadirachta indica* possess cytotoxic principles (LC₅₀ 1.429 µg/mL, 2.368 µg/mL and 1.479 µg/mL respectively) comparison with positive control vincristine sulphate (LC₅₀ 0.563 µg/mL).

INTRODUCTION: *Azadirachta indica* is a native tree of Bangladesh, found in every part in Bangladesh especially in semi-arid conditions. It is especially suited to semi-arid conditions and thrives even in the poorest soil with rainfalls as little as 18 inches (450 mm) per year and temperatures up to 50°C (120°F). It may grow up to 50 feet (15 m) tall and live for 200 years. The lifespan of the *Azadirachta indica* tree is described to be anywhere between 150 to 300 years. Its blossoms are small, white flowers with a very sweet, jasmine-like scent. Its edible fruit is about 3/4 of an inch (2 cm) long with white kernels.

Azadirachta indica tree generally begins bearing fruits at three to five years of age, and can produce up to 50 kg of fruit annually when mature. The pinnate leaves have a very bitter taste and a garlic-like smell.

Azadirachta indica had been used as a potential medicinal agent like anti-inflammatory^{1, 2}, anti-neoplastic activity⁵ melanogenesis inhibitory², immunostimulatory agent³, larvae of filaria⁴, tumor cell killing⁶, chemotactic activity⁷, larvae of *Boophilus decolotatus*⁹, acaricidal activity¹⁰, larvicidal activity^{11, 37}, anti-tumor activity^{13, 15, 17, 33}, leukocyte adopsis¹⁴, prophylactic activity¹⁶, anthelmintic activity^{18, 19}, hepatotoxicity²¹, anti-cancer effects²², transmission blocking activity²³, insecticidal activity²⁴, anti-oxidant activity²⁵, anti-trypanosomal²⁶, anti-hypertensive activity³¹, immunological function³², nutritional activity³⁴, free radical scavenging activity³⁵, antiviral activity³⁶, antibacterial activity³⁸, antimalarial activity³⁹, anti-diabetic⁴⁰ and anti-bovine activity⁴¹. Some potential



medicinal compounds had been isolated from *A. indica* like anti-plasmodial triterpenoids⁸ and snake venom phospholipase A2³⁰.

METHODS AND MATERIALS:

Collection and identification of the plant: Plant sample of *Azadirachta indica* root was collected from near the Noakhali Science and Technology University area in March 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 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.

Extraction of the plant material: The powdered roots (660 g) were soaked with methanol (2.5 L) in May, 2010 in a desicator through 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 greenish mass of biological investigation. The methanolic crude extract was fractionate according to Modified Kupchan partitioning method (figure 2) into *n*-hexane (1.0 g), ethyl acetate (1.5 g) and chloroform (0.7 g) soluble fractions (**figure 1**).

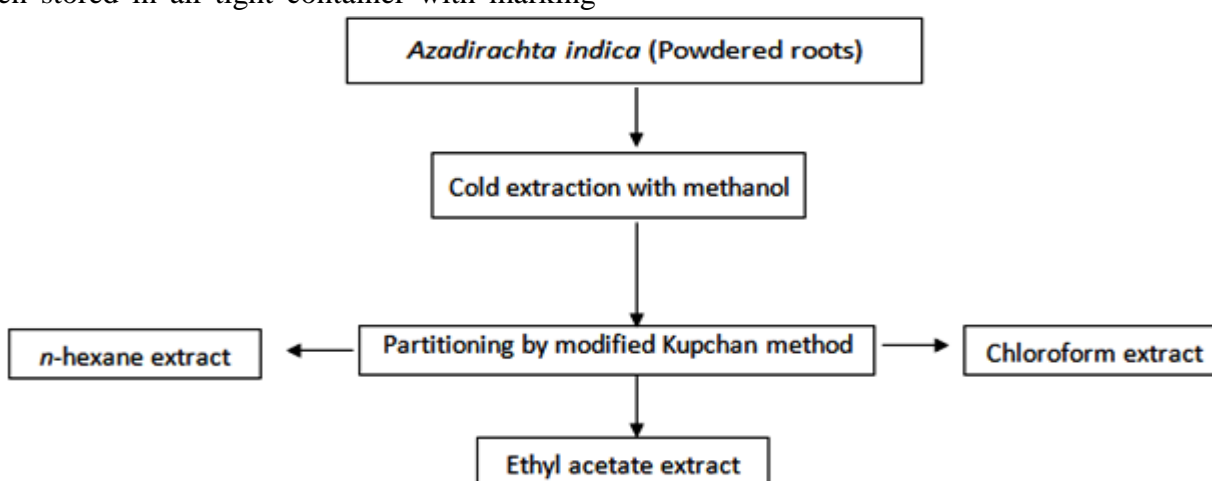


FIGURE 1: SCHEMATIC DIAGRAM OF THE CRUDE EXTRACTS OF AZADIRACHTA INDICA

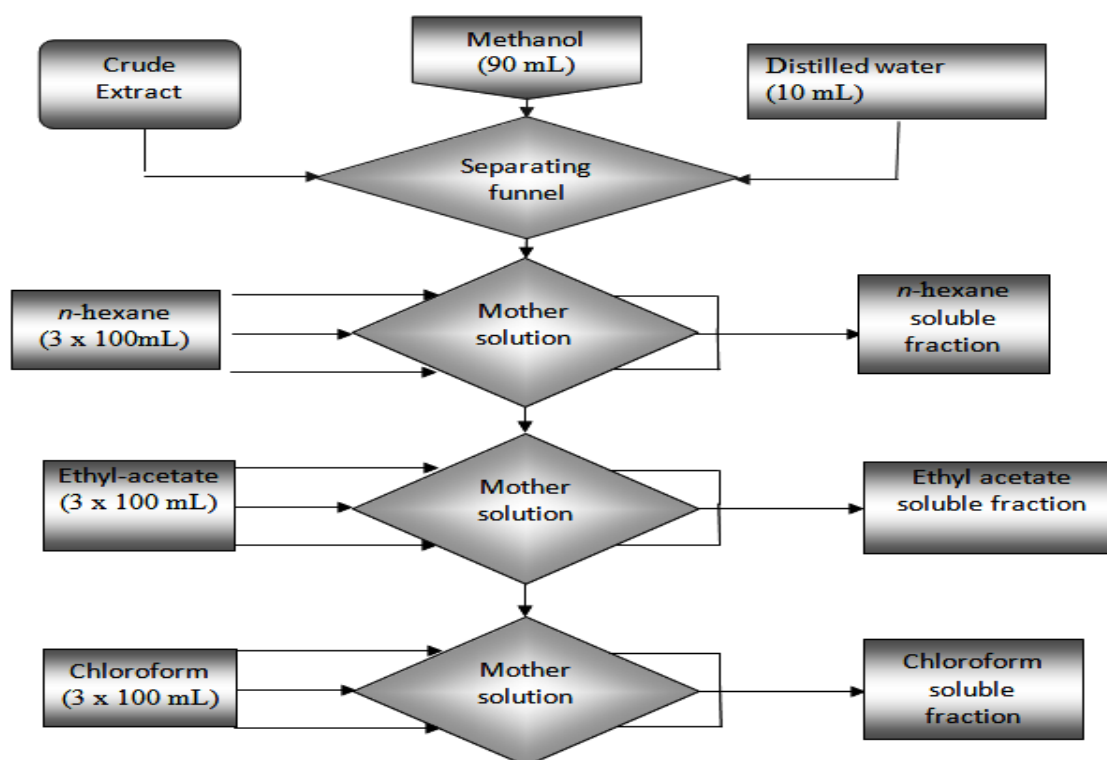


FIGURE 2: SCHEMATIC DIAGRAM OF A MODIFIED KUPCHAN PARTITIONING METHOD

RESULT AND DISCUSSION: The brine shrimp test represents a rapid, inexpensive and simple bioassay for testing plant extract lethality which in most cases correlates reasonably well with cytotoxic and anti-tumour properties²⁷. Following the procedure of Mayer²⁸, the lethality of the methanolic crude extract (n-hexane, ethyl acetate and chloroform fractions) were determined and the LC₅₀ values of n-hexane, ethyl acetate and chloroform soluble fraction found to be 1.429µg/mL 2.368µg/mL and 1.479

µg/mL respectively compared with the positive control vincristine sulphate (0.563µg/mL) (**Table 1**). From the results of the brine shrimp lethality bioassay it can be well predicted that the methanolic crude extracts (n-hexane, ethyl acetate and chloroform soluble fractions) possess cytotoxic properties. The effect of vincristine sulphate, n-hexane, ethyl acetate, and chloroform soluble fraction and are shown in **table 2 and figure 3, 4, 5 and 6**.

TABLE 1: RESULTS OF THE TEST SAMPLES OF AZADIRACHTA INDICA

Methanolic crude extracts	LC ₅₀ (µg/mL)	Regression equation	R ²
n-Hexane	1.429	y = 44.27x - 13.23	0.930
Ethyl acetate	2.368	y = 23.94x - 6.867	0.690
Chloroform	1.479	y = 43.06x - 13.71	0.925
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 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	80	100	1.429	2.368	1.479	40	1.602	100	0.563
200	2.301	100	60	90				20	1.301	90	
100	2	80	20	80				10	1.000	90	
50	1.699	70	10	60				5	0.698	80	
25	1.398	40	20	50				2.5	0.397	70	
12.5	1.097	20	20	20				1.25	0.096	70	
6.25	0.796	10	10	0				0.625	-0.204	50	
3.125	0.495	0	10	0				0.3125	-0.505	30	
1.56	0.193	0	0	0							
0.78	-0.108	0	0	0							

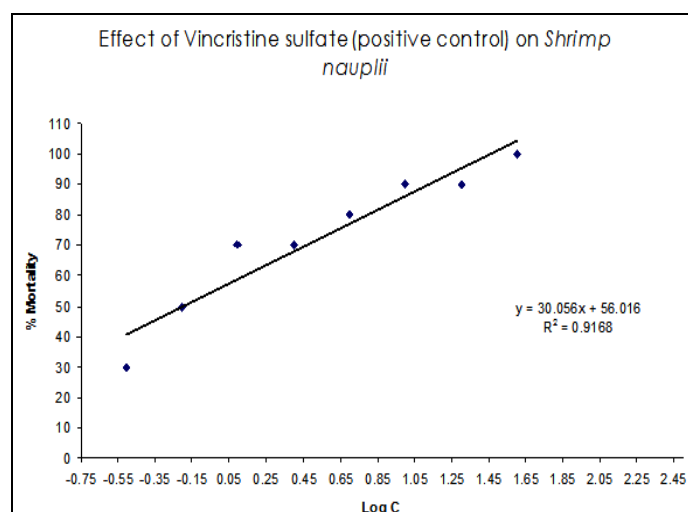


FIGURE 3: EFFECT OF VINCRIStINE SULPHATE ON BRINE SHRIMP NAUPLII

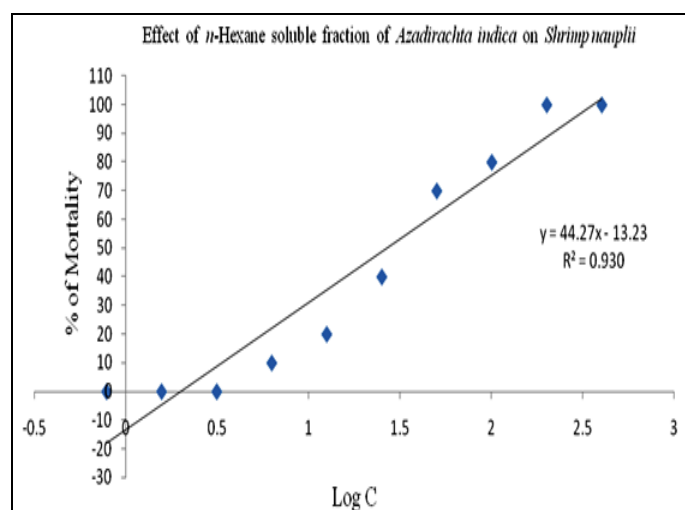


FIGURE 4: EFFECT OF N-HEXANE SOLUBLE FRACTION ON BRINE SHRIMP NAUPLII

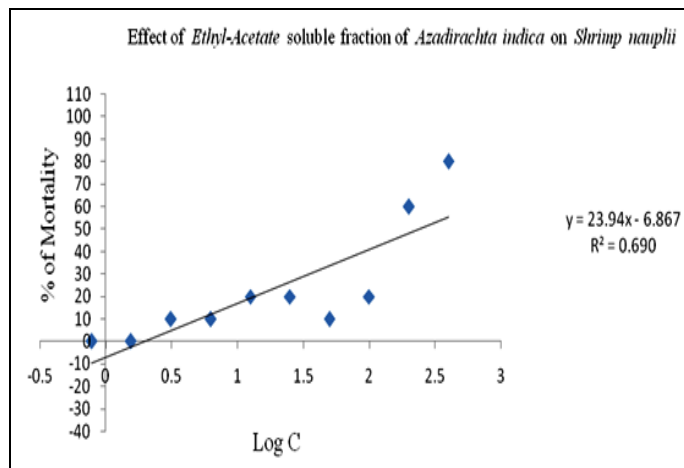


FIGURE 5: EFFECT OF ETHYL ACETATE SOLUBLE FRACTION ON BRINE SHRIMP NAUPLII

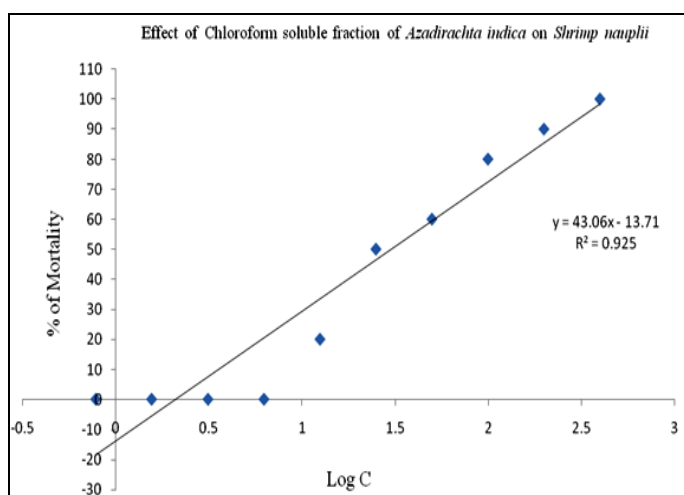


FIGURE 6: EFFECT OF CHLOROFORM SOLUBLE FRACTION ON BRINE SHRIMP NAUPLII

CONCLUSION: *Azadirachta indica* leaves and roots are good for blood circulation and blood purification. *Azadirachta indica* has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. *Azadirachta indica* elaborates a vast array of biologically active compounds that are chemically diverse and structurally complex.

More than 140 compounds have been isolated from different parts of *Azadirachta indica*. All parts of the *Azadirachta indica* tree- leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. *Azadirachta indica* leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, anti-hyperglycaemic, antiulcer, anti-malarial, antifungal, antibacterial, antiviral, anti-oxidant, antimutagenic and anticarcinogenic properties.

It comes out of my own personal experiences and study of the effects and properties of *Azadirachta indica*. It is also my conviction that *Azadirachta indica* is a wonderful cure for diseases and gives much to humans including shade, good air, health and overall well-being. I also think, it is important that people become aware of the values of *Azadirachta indica*. Among all the plants on this planet that have proved useful for humanity, a few are distinguished by their astonishing versatility.

Among these, the *Azadirachta indica* tree is one of the most important one. *Azadirachta indica* is deemed very effective in the treatment of scabies although only preliminary scientific proof exists which still has to be corroborated, and is recommended for those who are sensitive to permethrin, a known insecticide which might be irritant. Also, the scabies mite has yet to become resistant to *Azadirachta indica*, so in persistent cases *Azadirachta indica* has been shown to be very effective.

While *Azadirachta indica* products have some shortcomings as a conventional alternative, they fit in well as a tool to be used in integrated pest management systems. As more and more synthetic chemicals are being pulled from the market, *Azadirachta indica* is an environmentally benign alternative. It has significant effect on pests without harming beneficial organisms. Toxicology studies have indicated it to be quite safe to mammal's also. Researchers, however, still have much work ahead of them to characterize the responses of sensitive insects in the field.

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How to cite this article:

Rakib MMA and Hussain MM: Assessment of cytotoxic effect of Methanolic crude extracts of *Azadirachta indica*. *Int J Pharm Sci Res* 2013; 4(4); 1585-1590.