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IN VITRO CYTOTOXIC EFFECT OF METHANOLIC CRUDE EXTRACTS OF *OCIMUM SANCTUM*

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ABSTRACT: The aim of this research is to observe the cytotoxic activities of methanolic crude extracts of *Ocimum sanctum* (Family: Lamiaceae). The leaves of *Ocimum sanctum* was extracted with organic solvent (methanol) and the extracts were fractionated by using solvent-solvent partition. The n-hexane, ethyl acetate, and chloroform soluble fractions of methanolic crude extract of *Ocimum sanctum* 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 was well predicted that n-hexane, ethyl acetate, and chloroform soluble fractions of methanolic crude extracts possess cytotoxic principles (LC₅₀ 4.36 mg/mL, LC₅₀ 5.37 mg/mL and LC₅₀ 10.00 mg/mL respectively) comparison with positive control, vincristine sulphate (LC₅₀ 0.563 mg/mL).

INTRODUCTION: *Ocimum sanctum* (Local name: Tulsi, Family: Lamiaceae) is an aromatic plant which is native throughout the world tropics and widespread as a cultivated plant and an escaped weed. It is an erect, much branched subshrub 30-60 cm tall with hairy stems and simple opposite green leaves that are strongly scented. Leaves have petioles, and are ovate, up to 5 cm long, usually slightly toothed and flowers are purplish in elongate racemes in close whorls¹.

Ocimum sanctum has been used as a potent traditional medicinal agent in the antifungal², acetylcholinesterase and improve cognition condition³, immunomodulatory^{4, 13}, antiplasmodial^{5, 25}, radioprotective^{6, 14}, antidiabetic^{7, 15}, genoprotective⁸, hepatoprotective⁹, ameliorative¹⁰, larvicidal¹⁸, antioxidant^{19, 20, 22}, antityrosinase activity²⁰, reproductive²¹, lipid lowering²², antihyperlipidemic and cardioprotective²³ and anti-dengue activity²⁴.

METHODS AND MATERIAL:

Collection and identification of the plant: Plant sample of *Ocimum sanctum* was collected from Noakhali Science and Technology University campus in January 2010 and selected for cytotoxic activities.

Plant material preparation: The leaves of the plant were collected in fresh condition. It was sun-dried 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 plant material: The powdered leaves (700 g) were soaked in methanol (2.8 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 methanolic crude extract. The methanolic crude extract was fractionate according to Modified Kupchan partitioning method (**figure 2**) into n-hexane (0.04 g), ethyl acetate (0.6 g) and chloroform (0.06) soluble fractions.

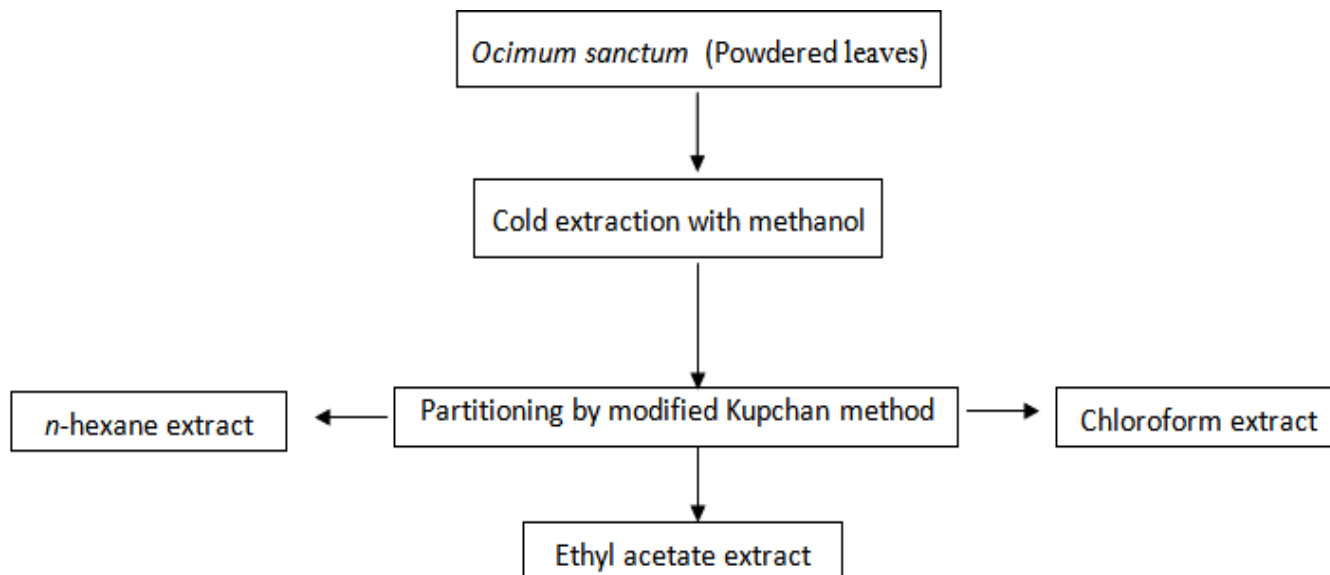


FIGURE 1: SCHEMATIC DIAGRAM OF THE CRUDE EXTRACTS OF *OCIMUM SANCTUM*

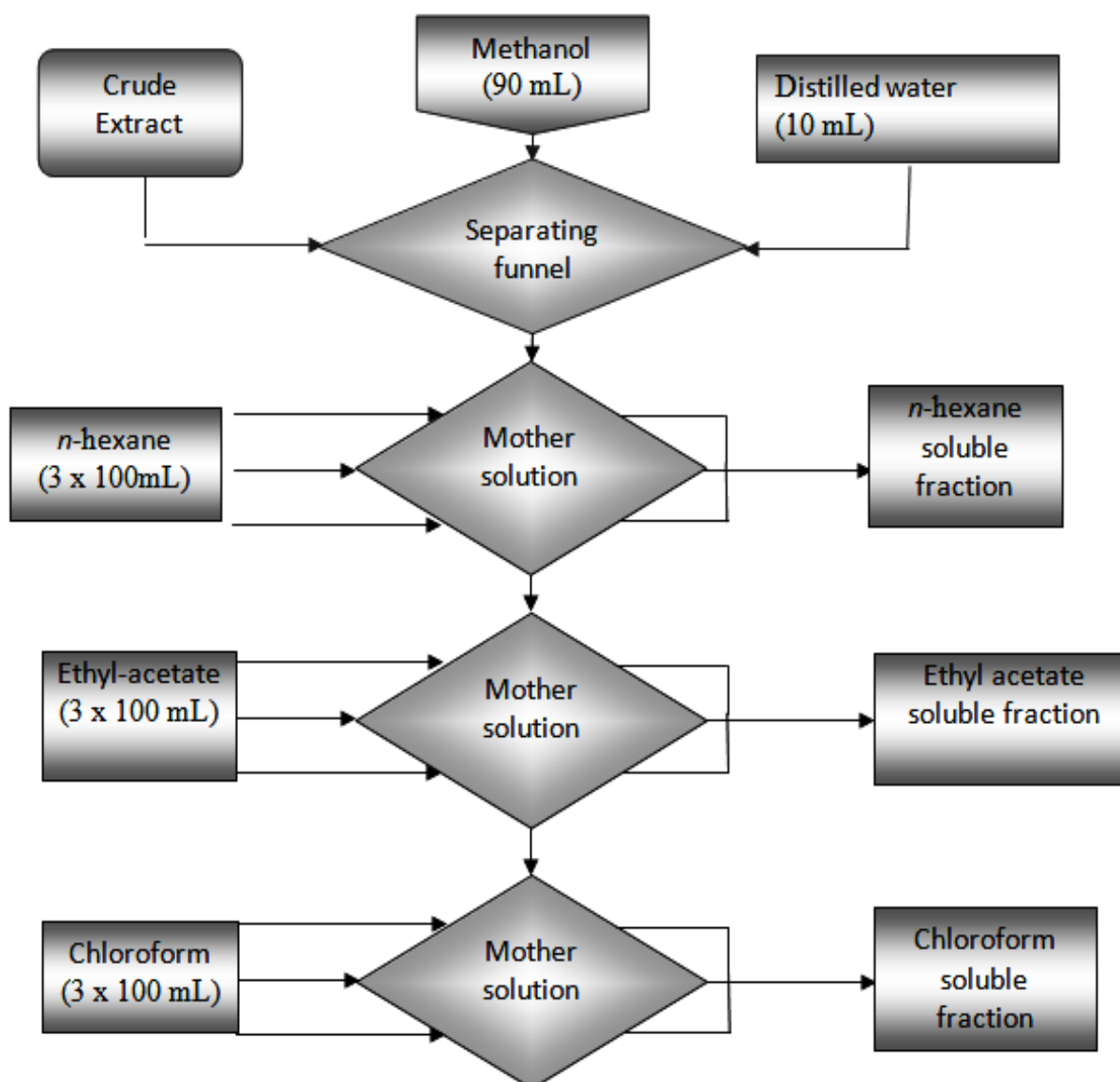


FIGURE 2: SCHEMATIC DIAGRAM OF A MODIFIED KUPCHAN PARTITIONING METHOD

RESULT AND DISCUSSION: The Brine Shrimp test (BST) represents a rapid, inexpensive and simple bioassay for testing cytotoxic and anti-tumor properties of plant extracts¹¹. Following the procedure of Meyer¹² and Persoone¹⁷, the cytotoxic effects of the 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 fractions found to be 4.365 mg/mL 5.370 mg/mL and

10.00 mg/mL respectively (**Table 1**) compared with positive control, vincristine sulphate (0.563 mg/mL). From the test it was observed that cytotoxic effects exhibited by the methanolic extract (n-hexane, ethyl acetate and chloroform soluble fractions). The effects of n-hexane, ethyl acetate, chloroform soluble fractions and vincristine sulphate on shrimp nauplii are shown in the **table 2** and **figure 3, 4, 5 and 6** respectively.

TABLE 1: LC₅₀ VALUES OF METHANOLIC CRUDE EXTRACTS OF OCIMUM SANCTUM

Methanolic crude extracts (soluble fractions)	LC ₅₀ (µg/mL)	Regression equation	R ²
n-hexane	4.365	y = 15.296x + 7.0786	0.5912
Ethyl acetate	5.370	y = 20.326x - 4.3527	0.3728
Chloroform	10.00	y = 19.522x - 9.3501	0.3295
Vincristine sulphate (positive control)	0.563	y = 30.056x + 56.016	0.9168

TABLE 2: EFFECT OF N-HEXANE, ETHYL ACETATE, CHLOROFORM SOLUBLE FRACTIONS AND VINCRISTINE SULPHATE ON BRINE SHRIMP NAUPLII

Conc. (C, µg/mL)	Log C	Mortality (%)			LC ₅₀ (µg/mL)			Vincristine sulphate			
		n-hexane	Ethyl acetate	Chloroform (CF)	n-hexane	Ethyl acetate	CF	Conc. (C, µg/mL)	Log C	Mortality (%)	LC ₅₀ (µg/mL)
400	2.602	60	10	100	4.365	5.370	10.0	40	1.602	100	0.563
200	2.301	20	30	0				20	1.301	90	
100	2	10	20	0				10	1.000	90	
50	1.699	10	0	20				5	0.698	80	
25	1.398	10	10	20				2.5	0.397	70	
12.5	1.097	10	30	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							
0.78	-0.108	0	20	0							

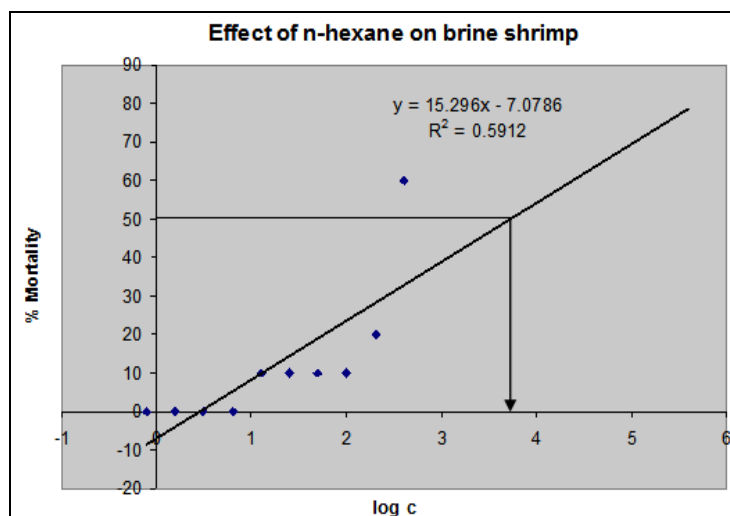


FIGURE 3: EFFECT OF N-HEXANE SOLUBLE FRACTION ON BRINE SHRIMP NAUPLII

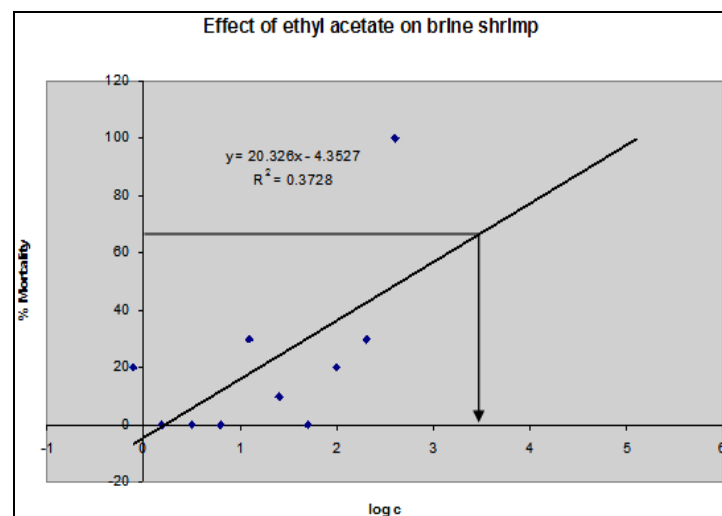


FIGURE 4: EFFECT OF ETHYL ACETATE SOLUBLE FRACTION ON BRINE SHRIMP NAUPLII

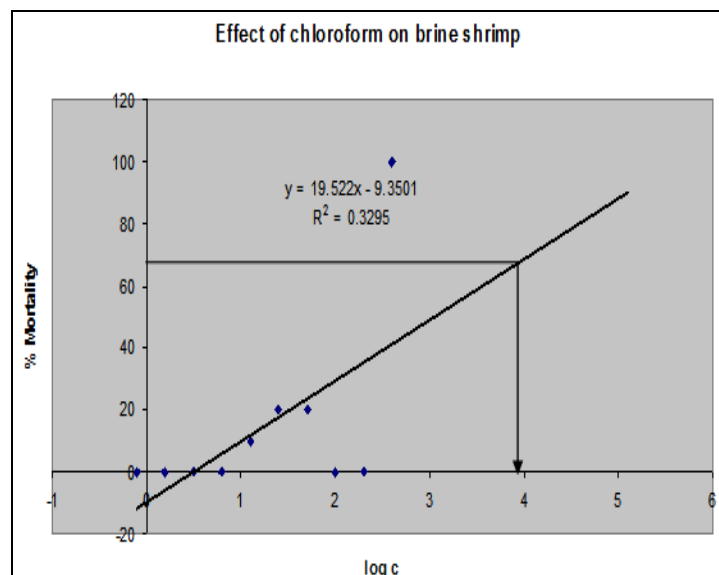


FIGURE 5: EFFECT OF CHLOROFORM SOLUBLE FRACTION ON BRINE SHRIMP NAUPLII

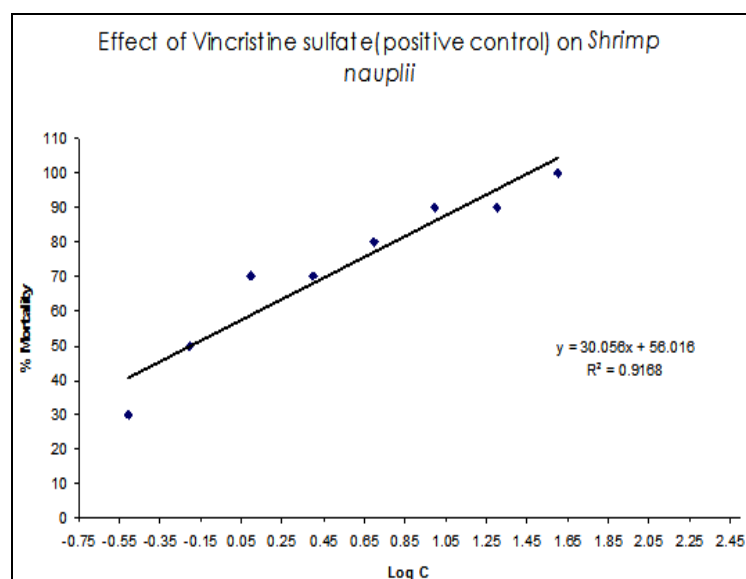


FIGURE 6: EFFECT OF VINCRISTINE SULPHATE ON BRINE SHRIMP NAUPLII

CONCLUSION: The present research indicates that the crude extracts of *Ocimum sanctum* has got intense *in-vitro* cytotoxic effect and may have potential use in traditional medicine.

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