IJPSR (2012), Vol. 3, Issue 06 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 21 January, 2012; received in revised form 29 March, 2012; accepted 17 May, 2012

CYTOTOXIC EFFECT OF CRUDE EXTRACTS OF *ACACIA NILOTICA*

Fahad Hussain [1] and Mohammad Musarraf Hussain*[2]

Department of Pharmacy, Noakhali Science and Technology University [1], Sonapur, Noakhali-3802, Bangladesh.
Department of Pharmacy, Jagannath University [2], Dhaka-1100, Bangladesh

Keywords:
Acacia nilotica,
Fabaceae
cytotoxic effects,
n-hehane,
ethyl acetate,
chloroform soluble fractions

Correspondence to Author:

Mohammad Musarraf Hussain

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

ABSTRACT

The cytotoxic effect of crude extracts (n-hehane, ethyl acetate and chloroform soluble fractions) of root of *Acacia nilotica* was observed by brine shrimp lethality bioassay. The LC_{50} values of n-hexane, ethyl acetate and chloroform soluble fraction found to be 1.956, 2.079 and 1.981 respectively indicating that the some of the compounds of the extract are biologically active. From this experiment, it was revelead that the test sample showed different response at different concentrations. The mortality rate of brine shrimp was found to be increased with the increased concentrations of sample, and a plot of log of concentration versus percent mortality on the graph produced an approximate linear correlation.

INTRODUCTION: *Acacia nilotica* (Family: Fabaceae, Local name: babul) is a tree 5-20 m high with a dense spheric crown, stems and branches usually dark to black coloured, fissured bark, grey-pinkish slash, exuding a reddish low quality gum.

The tree has thin, straight, light, grey spines in axillary pairs, usually in 3 to 12 pairs, 5 to 7.5 cm long in young trees, mature trees commonly without thorns. The leaves are bipinnate, with 3-6 pairs of pinnulae and 10-30 pairs of leaflets each, tomentose, rachis with a gland at the bottom of the last pair of pinnulae.

Flowers in globulous heads 1.2-1.5 cm in diameter of a bright golden-yellow color set up either axillary or whorly on peduncles 2-3 cm long located at the end of the branches. Pods are strongly constricted, hairy, white-grey, thick and softly tomentose. Its seeds number approximately 8000/kg [1, 18]. *Acacia nilotica* is being used as a potencial medicinal agent of anticancer [14], antimutagenic [11, 14], antibacterial [6], anti-inflammatory [6], kinase Inhibitor [2], free radical scavenging [10], anti-helmintic [5], antioxidant [17], anti-quorum sensing [17], larvicidal activity [7], cytotoxic effects [11], dose related contractile activity [3], anti-spasmodic activity [9], antihypertensive [9], molluscidal [4]. *A. nilotica* is a good sources of crude protein and mineral supplements to ruminants [16], a novel polyphenol, (+)-catechin-5-galloyl ester [12] and stimulate milk production and PRL release in the female rat [13].

METHODS AND MATERIALS:

Collection and *i*dentification of the plant*:* The plant sample (root) of *Acacia nilotica* was collected from Noakhali Science and Technology University campus in January, 2010.

Plant material preparation: The root of the plant was 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 plant material in powdered form (800 g) was soaked in methanol (2.30 L) in April 20, 2010 in a desecrator, kept on occasional stirring for 27days, filtered through filter-cloth and the filtrate was kept to the open air for evaporation of solvent to afford a crude extract of biological investigation.

Preparation of Mother Solution: The methanolic extract of *Acacia nilotica* root (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 of different polarity.

Partitioning with n-hexane: The mother solution was taken in a separating funnel. The solvent, n-hexane (100 mL) was added to it, funnel was shaken and then kept undisturbed. The organic portion was collected and the process was repeated in three times.

The organic phases (n-hexane fractions) were added together, solvent was evaporated in the air dry and the crud extract was found (0.60 gm).

Partitioning with Ethyl acetate: Distilled water (10 mL) was added with the mother solution left after washing with n-hexane. The mother solution was then taken into a separating funnel and extracted with ethyl acetate (3x100 mL). The ethyl acetate fractions were added together, solvent was evaporated in the dry air and extract was found (0.90 gm).

Partitioning with Chloroform: Distilled water (20 mL) was added with the mother solution that left after washing with n-hexane and ethyl acetate. The mother solution was then taken in a separating funnel and extracted with chloroform (3x100 mL). The chloroform soluble fractions were added together; solvent was evaporated in the air dry and extract was found (1.20 gm).

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-tumor properties. Following the procedure of Meyer [15] the cytotoxic effect (lethality) of the crude extracts of *Acacia nilotica*, n-hexane, ethyl acetate and chloroform fractions were determined and the summary of the result are expressed in **Table 1**.

The LC_{50} values of n-hexane, ethyl acetate and chloroform soluble fraction found to be 1.956, 2.079 and 1.981 respectively (**Table 2 and Figure 2, 3, 4**). The LC_{50} value for the positive control (vincristine sulphate) found to be -0.2001. 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 effect.

TABLE 1: RESULTS OF THE TEST SAMPLES OF *ACACIA NILOTICA*

Sample	LC_{50} (Based on Log C)	Regression equation	R^2
Vincristine sulphate (positive control)	-0.2001	y = 30.056x + 56.016	0.9168
n-Hexane soluble fraction	1.956	y = 33.98x - 16.47	0.570
Ethyl acetate soluble fraction	2.079	y = 32.54x - 17.67	0.517
Chloroform soluble fraction	1.981	y = 34.19x - 17.73	0.584

TABLE 2: EFFECT OF n-HEXANE, ETHYL ACETATE AND CHLOROFORM SOLUBLE FRACTION ON SHRIMP NAUPLII

Conc. (C) (µg/ml)	Log C	% Mortality			LC_{50} based on Log C			Vincristine Sulfate			
		n-Hexane	Ethyl acetate	Chloroform	*n*-Hexane	Ethyl acetate	Chloroform	Conc. (C) (µg/ml)	Log C	% Mortality	LC_{50} Based on Log C
400	2.602	100	100	100				40	1.602	100	
200	2.301	100	100	100				20	1.301	90	
100	2	40	20	30				10	1.000	90	
50	1.699	0	0	0				5	0.698	80	
25	1.398	0	0	10	1.956	2.079	1.981	2.5	0.397	70	-0.2001
12.5	1.097	0	0	0				1.25	0.096	70	
6.25	0.796	0	0	0				0.625	-0.204	50	
3.125	0.495	10	0	0				0.3125	-0.505	30	
1.56	0.193	10	0	10				0.156	-0.807	30	
0.78	-0.107	0	10	0				0.078	-1.108	10	

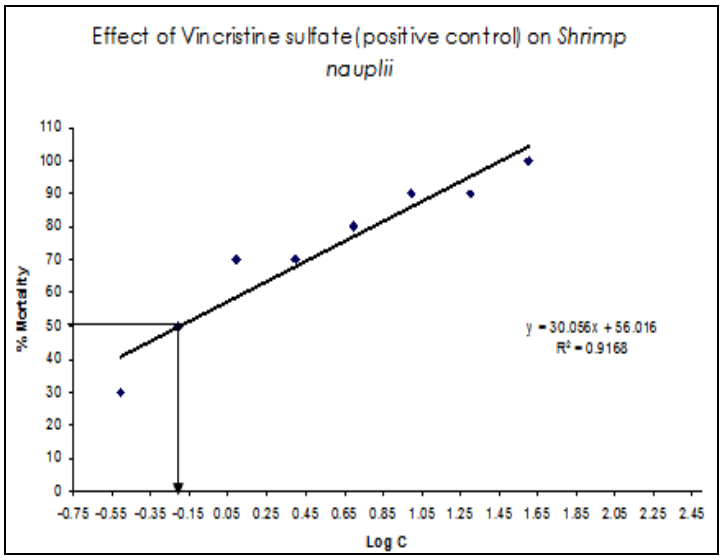

FIGURE 1: EFFECT OF VINCRISTINE SULPHATE ON BRINE SHRIMP NAUPLII

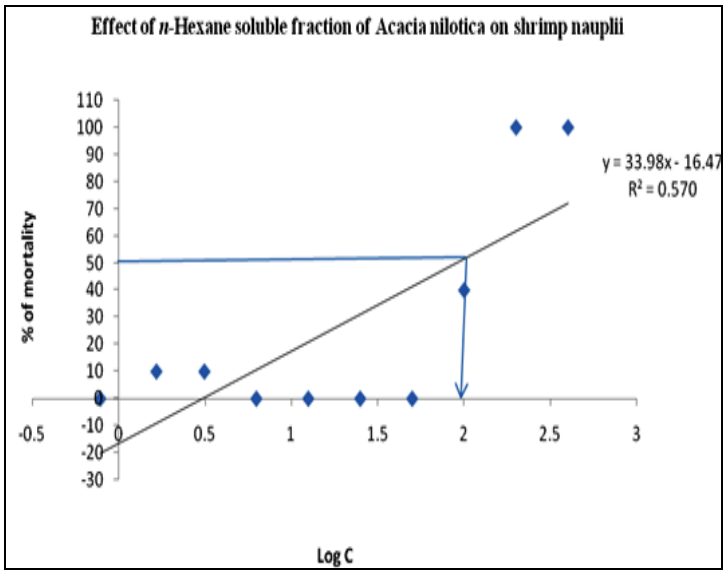

FIGURE 2: EFFECT OF n-HEXANE SOLUBLE FRACTION ON BRINE SHRIMP NAUPLII

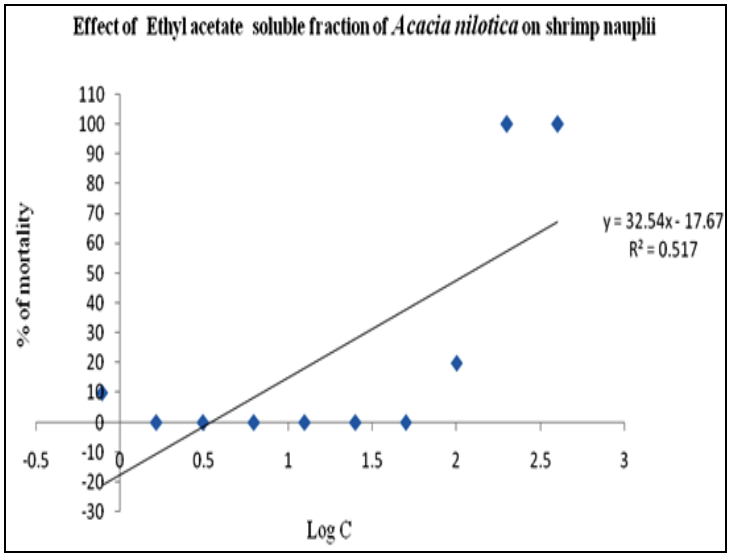

FIGURE 3: EFFECT OF ETHYL ACETATE SOLUBLE FRACTION ON BRINE SHRIMP NAUPLII

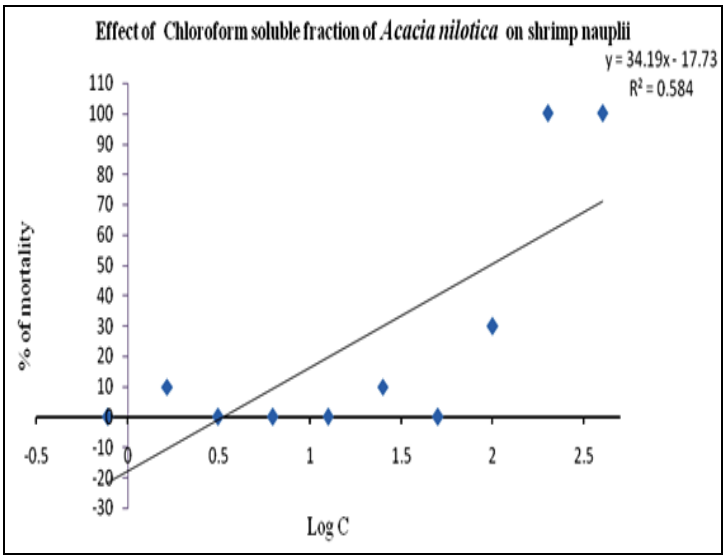

FIGURE 4: EFFECT OF CHLOROFORM SOLUBLE FRACTION ON BRINE SHRIMP NAUPLII

CONCLUSION: The present study indicates that the crude extracts of *Acacia nilotica* has 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.

REFERENCES:

1. Abdul Gani, Medicinal Plants of Bangladesh: Chemical Constituents and Uses, 1st edition, Asiatic Society of Bangladesh 1998.
2. Ahmadu A, Abdulkarim A, Grougnet R, Myrianthopoulos V, Tillequin F, Magiatis P, Skaltsounis AL; Two New Peltogynoids from *Acacia nilotica* Delile with Kinase Inhibitory Activity, Planta Med. 2009 Oct 20.
3. Amos S, Akah PA, Odukwe CJ, Gamaniel KS, Wambede C; The pharmacological effects of an aqueous extract from *Acacia nilotica* seeds, Phytotherapy Research, 1999, 13(8), 683-5.
4. Ayoub SM; Molluscicidal Properties of *Acacia nilotica*, Planta Medica, 1982, 46(11),181-3.
5. Bachaya HA, Iqbal Z, Khan MN, Sindhu ZU, Jabbar A; Anthelmintic activity of *Ziziphus nummularia* (bark) and *Acacia nilotica* (fruit) against Trichostrongylid nematodes of sheep, Journal of Ethnopharmacology, 2009, 23(2), 325-9.
6. Chaubal R, Mujumdar AM, Puranik VG, Deshpande VH, Deshpande NR; Isolation and X-ray study of an anti-inflammatory active androstene steroid from *Acacia nilotica*, Planta Med. 2003, 69(3), 287-8.
7. Chaubal R, Pawar PV, Hebbalkar GD, Tungikar VB, Puranik VG, Deshpande VH, Deshpande NR; Larvicidal activity of Acacia nilotica extracts and isolation of D-pinitol- a bioactive carbohydrate,Chem Biodivers. 2005, 2(5), 684-8.
8. Eldeen IM, Van Heerden FR, Van Staden J; *In vitro* biological activities of niloticane, a new bioactive cassane diterpene from the bark of *Acacia nilotica* subsp, Kraussiana, Journal of Ethnopharmacology, 2010 Feb 4.
9. Gilani AH, Shaheen F, Zaman M, Janbaz KH, Shah BH, Akhtar MS, Studies on antihypertensive and antispasmodic activities of

methanol extract of *Acacia nilotica* pods, Phytotherapy Research, 1999, 13(8), 665-9.

10. Kalaivani T, Mathew L; Free radical scavenging activity from leaves of *Acacia nilotica* (L.) Wild. Ex Delile, an Indian medicinal tree, Food Chem Toxicology, 2010, 48 (1), 298-305.
11. Kaur K, Michael H, Arora S, Härkönen P, Kumar S; *In vitro* bioactivity-guided fractionation and characterization of poly phenolic inhibitory fractions from *Acacia nilotica* (L.) Willd. Ex Del, Journal of Ethnopharmacology, 2005, 99(3), 353-60.
12. Khalid SA, Yagi SM, Khristova P, Duddeck H; (+)-Catechin-5-galloyl Ester as a Novel Natural Polyphenol from the Bark of *Acacia nilotica* of Sudanese Originl, Planta Med. 198, 55(6), 556-8.
13. Lompo-Ouedraogo Z, van der Heide D, van der Beek EM, Swarts HJ, Mattheij JA, Sawadogo L; Effect of aqueous extract of *Acacia nilotica* ssp adansonii on milk production and prolactin release in the rat, Journal of Endocrinology, 2004,182(2), 257-66.
14. Meena PD, Kaushik P, Shukla S, Soni AK, Kumar M, Kumar A; Anticancer and antimutagenic properties of *Acacia nilotica* (Linn.) on 7, 12-dimethylbenz(a) anthracene-induced skin papillomagenesis in Swiss albino mice, Asian Pacific Journal of Cancer Preview, 2006, 7(4):627-32.
15. Meyer, B. N, Ferringni, N, R., Puam, J, E., Lacobsen, L, B., Nichols, D.E. and McLaughlin, J. L.; Brine shrimp: a convenient general bioassay for active constituents, Planta Medica. 1982, 45, 31-32.
16. Rubanza CD, Shem MN, Bakengesa SS, Ichinohe T, Fujihara T; The content of protein, fibre and minerals of leaves of selected *Acacia* species indigenous to north-western Tanzania, Arch Anim Nutrition, 2007, 61(2), 151-6.
17. Singh BN, Singh BR, Singh RL, Prakash D, Sarma BK, Singh HB, Antioxidant and anti-quorum sensing activities of green pod of *Acacia nilotica* L, Food Chem Toxicology, 2009, 47(4), 778-86.
18. Yusuf, M., Chowdhury, J.U., Wahab, M.A. and Begum, J, Medicinal Plants of Bangladesh, Bangladesh Council of Scientific & Industrial Research (BCSIR), Dhaka, Bangladesh, 1994.

****