



Received on 16 June, 2011; received in revised form 19 October, 2011; accepted 05 November, 2011

## ANTIMICROBIAL SCREENING AND BRINE SHRIMP LETHALITY BIOASSAY OF *TINOSPORA CORDIFOLIA* (FAM: MENISPERMACEAE)

Mohammad Kaiserul Islam\* and Khondaker Ashakin

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh  
Phytochemical Research Laboratory, Department of Pharmacy, University of Asia Pacific, Dhaka-1209, Bangladesh

### ABSTRACT

#### Keywords:

*Tinospora cordifolia*,  
Disc Diffusion Method,  
Brine Shrimp Lethality Bioassay

#### Correspondence to Author:

**Mohammad Kaiserul Islam**

Department of Pharmaceutical &  
Biological Chemistry, School of Pharmacy,  
University of London, 29-39 Brunswick  
Square, London WC1N 1AX, United  
Kingdom

*Tinospora cordifolia* (Fam: *Menispermaceae*) has been investigated for evaluation of the biological activities. The stem of *Tinospora cordifolia* were extracted with carbon tetrachloride, n-hexane and methanol were collected and afford 4.0 mg, 4.0 mg, and 4.0 mg, respectively for the test. The crude carbon tetrachloride, n-hexane, and methanol extract of the plant were subjected to antimicrobial screening against 15 microorganisms such as gram-positive, gram-negative, fungi by the disc diffusion method. But interestingly no fraction showed any inhibitory effect against all the microorganisms. In the brine shrimp lethality bioassay, carbon tetrachloride fraction showed high toxicity, where LC<sub>90</sub> value was only 6.25 µg/ml. The methanol crude extract and n-hexane fraction showed LC<sub>50</sub> of 1.563 and 6.05 µg/ml respectively with very narrow therapeutic index (LC<sub>90</sub> of 43.0 µg/ml and 45.0 µg/ml respectively). This indicated that the cytotoxicity exhibited by the carbon tetra chloride, n-hexane, and methanol extract was very significant.

**INTRODUCTION:** Plants have a great potential for producing new drugs for human benefit. Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and even infectious diseases. According to World Health Organization, more than 80% of world's populations depend on traditional medicine for their primary health care needs.

The demand for more and more drugs from plant sources is continuously increasing. It is therefore essential for systematic evaluation of plants used in traditional medicine for various ailments. In our study, we chose *Tinospora cordifolia*, (Bengali name - Gulancha) which is a glabrous, succulent, climbing shrub native to India. It is also found in Burma and Sri Lanka <sup>1, 2</sup>. *Tinospora cordifolia* is a large, climbing

shrub. A deciduous unknown that grows to 1.0 meters (3.3 feet) high by 0.5 meters (1.65 feet) wide and prefers many types of soil ranging from acid to alkaline and partial to full sun with moderate moisture. This plant has hermaphrodite flowers <sup>1-4</sup>. An aqueous extract of *T. cordifolia* decreased bronchospasm in guinea pigs, decreased capillary permeability in mice and reduced the number of disrupted mast cells in rats; a formulation containing *Tinospora cordifolia* markedly inhibited the suppression of chemotactic activity <sup>5, 6</sup> and production of interleukin-I and tumor necrosis factor induced by the carcinogen ochratoxin in mouse macrophages, further these extract of *T. cordifolia* showed antineoplastic activity, antiulcer activity, immunomodulatory activity, hypoglycemic activity, cholesterol-lowering activity and liver-protective activity <sup>5-13</sup>.

In the present, experiment an attempt has been made to evaluate the antibacterial activity of different extracts (n-hexane, carbon tetrachloride and methanol crude extract) against twelve prominent gram-positive and gram-negative human pathogenic bacteria and three fungal strains. Besides, cytotoxic activity screening of the extracts was also carried out with view to assess the presence of antitumor activity of different extracts.

## MATERIALS AND METHODS:

**Plant Materials:** Stem sample of *Tinospora cordifolia* was collected from an ayurvedic shop of Dhaka, New Market, on July 2008 and was identified by a Taxonomist of Bangladesh National Herbarium (BNH). The sample was then oven dried for 24 hours at considerably low temperature to effect grinding. The seeds were then ground into a coarse powder. The air-dried and powdered plant material (200.5 gm) was extracted with methanol. The time duration was of 21 days at room temperature with occasional shaking and stirring for each successive extraction. It was then filtered through a fresh cotton plug and finally with a Whatmann filter paper no. 1. The volume of the filtrate was then reduced using a Buchii Rotavapor at low temperature and pressure. It was then air-dried and powdered with crushing machine. Then the powdered material was successively extracted with methanol by using cold extraction process<sup>14</sup>. The crude extract was then fractionated into n-hexane and carbon tetrachloride by using kupchan partitioning method<sup>15</sup>.

**Methods for cytotoxic study:** 4.0 mg of each fraction (n-hexane, carbon tetrachloride and methanol) of *Tinospora cordifolia* were taken and dissolved in 200 $\mu$ l of pure dimethyl sulfoxide (DMSO) in two vials to get stock solutions. A series of solutions of different concentrations were prepared from the stock solution by serial dilution method and the concentrations were as; 400 $\mu$ g/ml, 200 $\mu$ g/ml, 100 $\mu$ g/ml, 50 $\mu$ g/ml, 25 $\mu$ g/ml, 12.5 $\mu$ g/ml, 6.25 $\mu$ g/ml, 3.125 $\mu$ g/ml, 1.5625 $\mu$ g/ml and 0.78125 $\mu$ g/ml. Then, the samples were subjected to brine shrimp lethality bioassay<sup>16, 17</sup> for cytotoxic studies. In each test tube, containing different concentrations of test sample, 10 brine shrimp nauplii (*Artemia salina*) were added. Two control groups were used in cytotoxicity study, to validate the test method and results obtained due to the activity of the test

agent. In the study vincristine sulphate was used as the positive control. Measured amount of the vincristine sulphate was dissolved in DMSO to get an initial concentration of 20  $\mu$ g/ml and serial dilutions were made using DMSO to get 10  $\mu$ g/ml, 5  $\mu$ g/ml, 2.5 $\mu$ g/ml, 1.25  $\mu$ g/ml, 0.625  $\mu$ g/ml, 0.3125  $\mu$ g/ml, 0.15625  $\mu$ g/ml, 0.078125  $\mu$ g/ml and 0.0390  $\mu$ g/ml of concentration. 30  $\mu$ l of DMSO was added to each of three premarked glass vials containing 5 ml of simulated seawater and 10 shrimp nauplii to use as negative control groups. After 24 hours, the test tubes were observed and the numbers of survived nauplii in each test tube were counted and the results were noted. From this, the percentage of lethality of brine shrimp nauplii was calculated at each concentration for each sample.

**Methods for antimicrobial assay:** Collected all fractions, i.e., n-hexane, carbon tetrachloride and methanol extracts were tested for antimicrobial study by using standard disc diffusion method<sup>18, 19</sup>. In this study, 16 microorganisms were obtained from the Institute of Nutrition and Food Sciences (INFS), University of Dhaka, Bangladesh. Standard Kanamycin (30  $\mu$ g/disc) and blank sterile filter paper disc (diameter, 6 mm) were used as positive and negative controls, respectively. Nutrient agar medium (DIFCO) was used in the present study for testing the sensitivity of the organisms to the test materials and to prepare fresh cultures. The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates, pre-inoculated with test bacteria. The discs were then incubated on the plate aerobically at 37 $^{\circ}$ C for 24 hours. The diameter of inhibition zone around each disc was measured and recorded at the end of the incubation period.

## RESULTS AND DISCUSSION:

**Antimicrobial study:** From the study, it was amazingly found that no zones of inhibition produced by the methanol extract, n-hexane and carbon tetrachloride fractions at a concentration of 400  $\mu$ g/disc in case of 12 bacterial strains and 03 fungal strains where standard doxycycline (30 $\mu$ g/disc) showed zone of inhibition of 43 - 52 mm (**Table 1**). The study was done for two times for the confirmation of no inhibitory effect.

TABLE 1: ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF DIFFERENT EXTRACTS OF *T. CORDIFOLIA*

Test organism	Diameter of Zone of Inhibition (mm)			
	Methanol extract 400 µg/disc	N-Hexane fraction 400 µg/disc	CCl <sub>4</sub> fraction 400 µg/disc	Doxycycline 30 µg/disc
<b>Gram positive bacteria</b>				
<i>Bacillus cereus</i> (BTCC-19)	na	na	na	50
<i>Bacillus megaterium</i> (BTCC-18)	na	na	na	50
<i>Bacillus subtilis</i>	na	na	na	46
<i>Staphylococcus aureus</i> (BTCC-43)	na	na	na	46
<i>Sarcina lutea</i> (ATCC-9341)	na	na	na	50
<b>Gram negative bacteria</b>				
<i>Escherichia coli</i> (BTCC-172)	na	na	na	49
<i>Salmonella paratyphi</i>	na	na	na	43
<i>Salmonella typhi</i>	na	na	na	51
<i>Shigella boydii</i>	na	na	na	50
<i>Shigella dysenteriae</i>	na	na	na	50
<i>Vibrio mimicus</i>	na	na	na	52
<i>Vibrio parahaemolyticus</i>	na	na	na	50
<b>Fungi</b>				
<i>Saccharomyces cerevisiae</i>	na	na	na	50
<i>Candida albicans</i>	na	na	na	50
<i>Aspergillus niger</i>	na	na	na	49

(na = No activity)

**Cytotoxic study:** In the present bioactivity study, all of the extracts (n-hexane, carbon tetrachloride and methanol) showed positive results indicating that the test samples are biologically active. Plotting of log of concentration (log C) versus percent mortality (% Mortality) for all test samples showed an approximate linear correlation. From the graphs, the median lethal concentration (LC<sub>50</sub>, the concentration at which 50% mortality of brine shrimp nauplii occurred) were determined and LC<sub>90</sub> values were also determined to establish the therapeutic index.

The methanol and n-hexane and fraction of *T. cordifolia* showed significant cytotoxic activity against brine shrimp nauplii and LC<sub>50</sub> value was found to be 1.563 µg/ml and 6.05 µg/ml (Table 2, Figure 1 & 2)

respectively whereas the very toxic effect was found for carbon tetrachloride fraction (LC<sub>90</sub> = 6.25 µg/ml). To get the therapeutic index, LC<sub>90</sub> (90% mortality) values were calculated for all fractions and the values were 43.0 µg/ml and 45.0 µg/ml (Table 2, Figure 1, 2 & 3) for methanol extract and n-hexane fraction respectively. For the conformity of the result, the test was done for two times.

An approximate linear correlation was observed when logarithm of concentration versus percentage of mortality was plotted on the graph paper and the values of LC<sub>50</sub> and LC<sub>90</sub> were calculated using Microsoft Excel 2007. All the values were compared with standard cytotoxic agent, vincristine sulphate who's LC<sub>50</sub> was found to be 0.625µg/ml.

TABLE 2: BRINE SHRIMP LETHALITY BIOASSAY OF *T. CORDIFOLIA*

Conc. (µg/ml)	LogC	% Mortality			LC <sub>50</sub> (µg/ml)			LC <sub>90</sub> (µg/ml)		
		ME	Hex	CTC	ME	Hex	CTC	ME	Hex	CTC
400	2.602	100	100	100						
200	2.301	100	100	100						
100	2.000	100	100	100						
50	1.699	100	100	100						
25	1.398	80	70	100						
12.5	1.097	80	60	100	1.563	6.05	Beyond Limit	43.0	45.0	6.25
6.25	0.796	70	50	90						
3.125	0.495	60	30	80						
1.563	0.194	50	20	70						
0.781	- 0.107	40	20	70						

(ME = methanol extract, Hex = n-hexane fraction, CTC = carbon tetrachloride fraction)

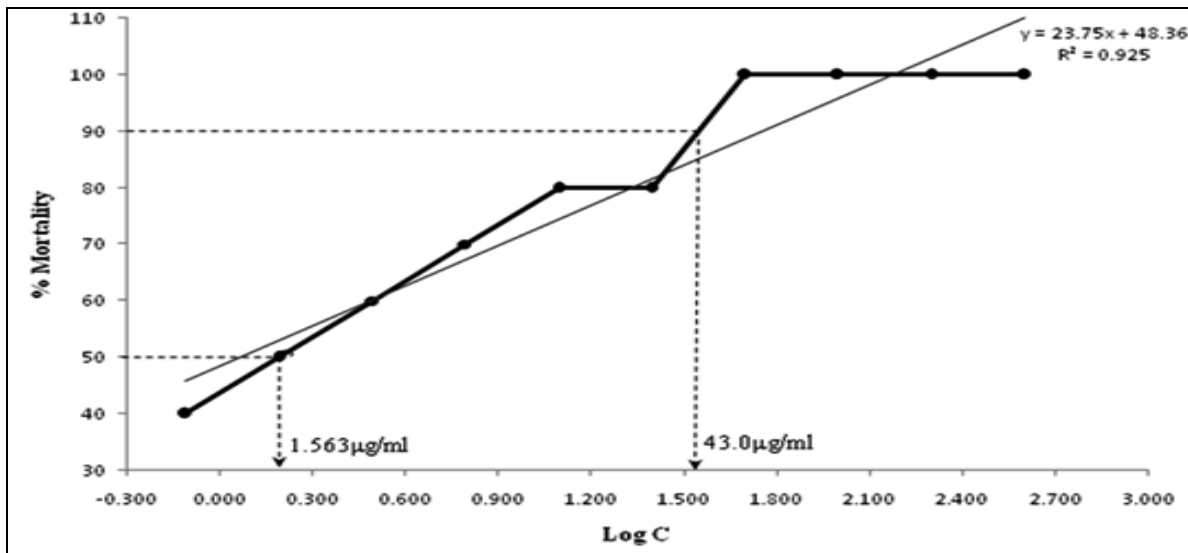


FIG. 1: DETERMINATION OF LC<sub>50</sub> AND LC<sub>90</sub> OF METHANOLIC EXTRACT OF *T. CORDIFOLIA*

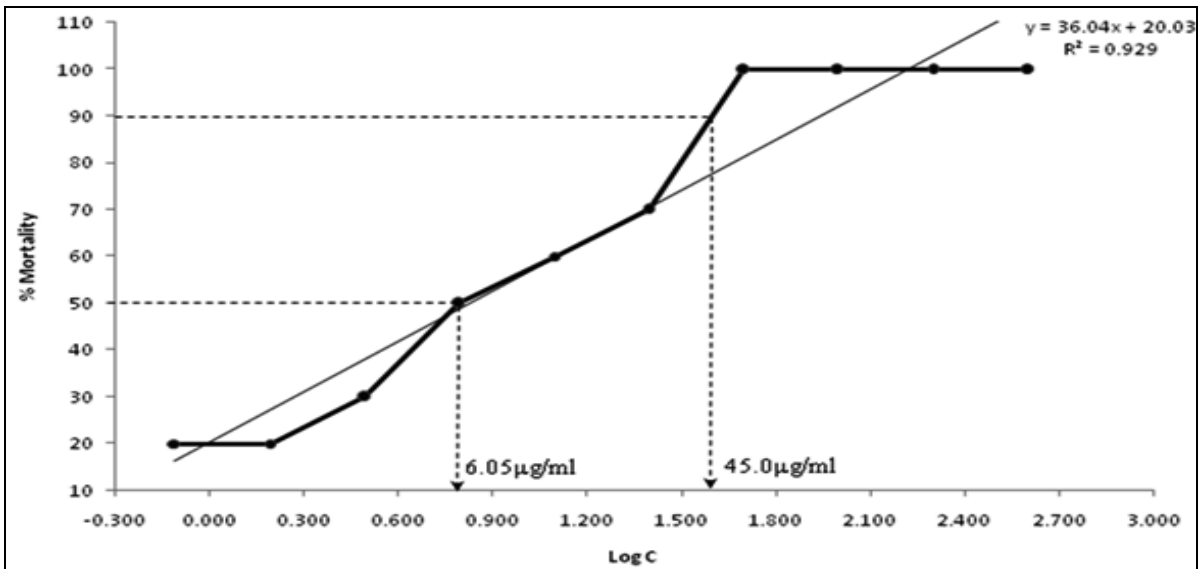


FIG. 2: DETERMINATION OF LC<sub>50</sub> AND LC<sub>90</sub> OF N-HEXANE FRACTION OF *T. CORDIFOLIA*

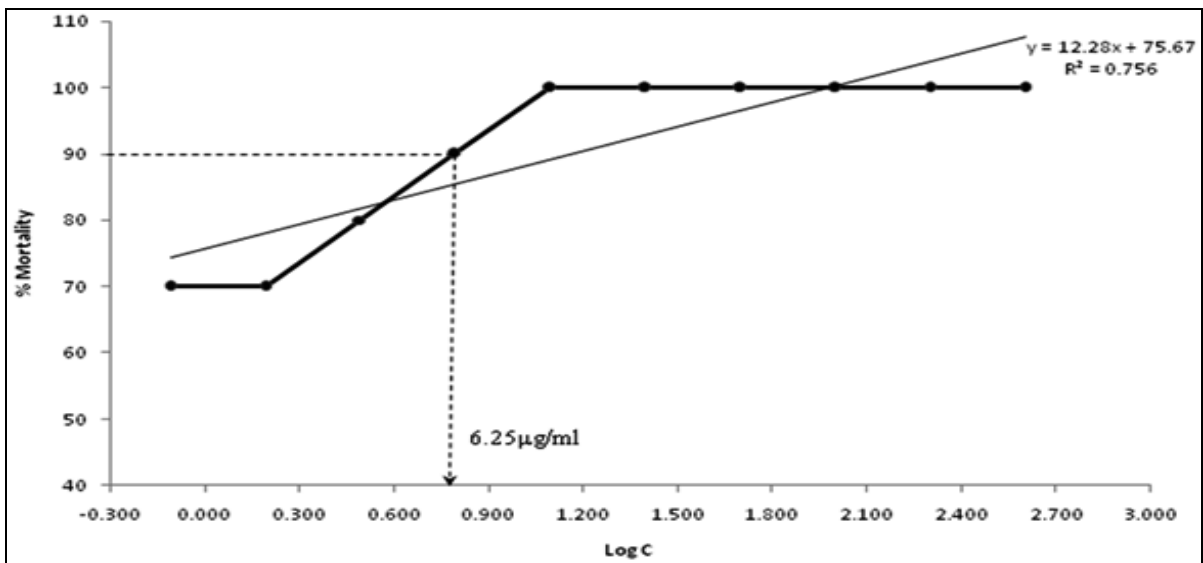


FIGURE 3: DETERMINATION OF LC<sub>50</sub> AND LC<sub>90</sub> OF CARBON TETRACHLORIDE FRACTION OF *T. CORDIFOLIA*

**ACKNOWLEDGEMENTS:** The authors are thankful to Institute of Nutrition and Food Sciences (INFS), University of Dhaka, Bangladesh for supplying the bacterial and fungal strains and Bangladesh National Herbarium (BNH) for identifying the plant.

#### REFERENCE:

1. Wagner, H. Immunomodulatory agents from plants. Birkhäuser 1999; 294. (ISBN 9783764358488).
2. Warriar, PK., Nambiar, VPK, Ramankutty, C. and Nair, RV. Indian medicinal plants: a compendium of 500 species. Orient Blackswan 1996; 5: 283.
3. Najib Nik A, Rahman N, Furuta T, Kojima S, Takane K, Ali Mohd M. Antimalarial activity of extracts of Malaysian medicinal plants. J Ethnopharmacol 1999; 649(3): 249–54.
4. Singh, SS., Pandey, SC., Shrivastava, S., Gupta VS and Palio, B. Chemistry and medicinal properties of *Tinospora cordifolia*. Indian J Pharmacol 2003; 35: 83–89.
5. Singh, N., Singh, SM. and Shrivastava, P. Immunomodulatory and antitumor actions of medicinal plant *Tinospora cordifolia* are mediated through activation of tumor-associated macrophages. Immunopharmacol Immunotoxicol. 2004; 26: 145-62.
6. Singh, N., Singh, SM. and Shrivastava, P. Effect of *Tinospora cordifolia* on the antitumor activity of tumor-associated macrophages-derived dendritic cells. Immunopharmacol Immunotoxicol. 2005; 27: 1-14.
7. Bishayi, B., Roychowdhury, S. and Ghosh, S. Hepatoprotective and immunomodulatory properties of *Tinospora cordifolia* in CCl<sub>4</sub> intoxicated mature albino rats. J Toxicol Sci. 2002; 27: 139-146.
8. Nair, PK., Rodriguez, S. and Ramachandran, R. Immune stimulating properties of a novel polysaccharide from the medicinal plant *Tinospora cordifolia*. Int Immunopharmacol. 2004; 4: 1645-1659.
9. Rathi, SS., Grover, JK and Vikrant, V. Prevention of experimental diabetic cataract by Indian Ayurvedic plant extracts. Phytother Res. 2002; 16: 774-777.
10. Rawal, AK, Muddeshwar, MG. and Biswas, SK. *Rubia cordifolia*, *Fagonia cretica* linn and *Tinospora cordifolia* exert neuroprotection by modulating the antioxidant system in rat hippocampal slices subjected to oxygen glucose deprivation. BMC Complement Altern Med. 2004; 4: 11.
11. Stanely, P., Prince, M. and Menon, VP. Hypoglycaemic and other related actions of *Tinospora cordifolia* roots in alloxan-induced diabetic rats. J Ethnopharmacol. 2000; 70: 9-15.
12. Stanely, MPP and Menon, VP. Antioxidant action of *Tinospora cordifolia* root extract in alloxan diabetic rats. Phytother Res. 2001; 15: 213-218.
13. Stanely, MPP and Menon, VP. Hypoglycaemic and hypolipidaemic action of alcohol extract of *Tinospora cordifolia* roots in chemical induced diabetes in rats. Phytother Res. 2003; 17: 410-413.
14. Trease, GE. And Evans, WC. Trease and Evans' Pharmacognosy, 13th ed. London; Philadelphia: Baillire Tindall. 1989.
15. Vanwagenen, BC, Larsen, R., Cardellina, JH. II., Randazzo, D., Lidert, ZC and Swithenbank, C J. Ulosantoin, a potent insecticide from the sponge *Ulosa ruetzleri*. Org. Chem. 1993; 58: 335-337.
16. Meyer B.B, Ferringi N.R, Futman F.J, Jacobsen L.B, Nichols D.E, McLaughlin J.L: Planta Medica 1982; 5: 31-34.
17. McLaughlin, JL., Chang, CJ. and Smith, DL. Frontiers in Natural Products Chemistry, Shamim Printing Press, Karachi 1990; 550-553.
18. Murray, PR., Baron, EJ, Pfaller, MA, Tenover, FC and Tenover, FC. Manual of Clinical Microbiology, 6th ed. Vol-6, Washington DC 1995; 214-215.
19. Zavala, SMA, Perez, GS and Perez, GM. Antimicrobial screening of some medicinal plants. Phytotherapy Res. 1997; 11: 368– 371.

\*\*\*\*\*