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# ANTIMICROBIAL AND CYTOTOXIC ACTIVITIES OF *TINOSPORA CORDIFOLIA* (FAM: MENISPERMACEAE)

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## ABSTRACT

*Tinospora cordifolia,* belonging to the Menispermaceae family, has been investigated for isolation of its secondary metabolites and evaluation of biological activities with special emphasis to the antimicrobial screening and cytotoxic study. The crude methanolic extract of *Tinospora cordifolia* along with its *Pet ether*, carbon tetrachloride, and chloroform soluble fractions were subjected to microbiological investigation by the disk diffusion method. The $CCl_4$ soluble fraction was found to have mild to moderate anti-microbial action. In the brine shrimp lethality bioassay, the *Pet ether*, $CCl_4$, chloroform and aqueous soluble fractions of crude methanolic extract were found to show $LC_{50}$ 1.918 μg/ml, 0.402μg/ml, 0.691 μg/ml and 2.246 μg/ml respectively. This indicated that the carbon tetrachloride and chloroform fractions were highly cytotoxic.

**INTRODUCTION:** The plant under investigation *Tinospora cordifolia* also called Gulancha/Guduchi in Bangla belongs to the family menispermaceae is an herbaceous vine. The plant is a glabrous climbing shrub found throughout India, Myanmar and Sri Lanka and Bangladesh typically growing in deciduous and dry forests [1, 2].

Recent research has demonstrated that a combination of *Tinospora cordifolia* extract and turmeric extract is effective in preventing the hepatotoxicity which is otherwise produced as a side effect of conventional pharmaceutical treatments for tuberculosis using drugs such as isoniazid and rifampicin [3].

**MATERIALS AND METHODS:**

**Collection of plant materials:** Plant sample of *Tinospora cordifolia* was collected from Curzon Hall area, Dhaka in June 2008. One voucher specimen has been deposited in Bangladesh National Herbarium and another one to Dhaka University Herbarium.

**Preparation of crude extract:** Only dried stem portion of *Tinospora cordifolia* was soaked in methanol for 15 days. A portion (5g) of the concentrated methanol extract was fractionated by the modified Kupchan partitioning method into pet. ether, carbontetrachloride, chloroform and aqueous soluble fractions [4]. Evaporation of solvent yielded Pet. ether extract (2.46gm), Carbontetrachloride Extract (1.20gm) Chloroform extract (174mg).

**Antibacterial screening:** The antimicrobial activity of the Kupchan fractions was determined by the disc diffusion method [5]. For this purpose thirteen bacterial strains and 3 fungi (listed in table 1) were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. The samples were dissolved separately in chloroform and applied to sterile discs at a concentration of 400µg/disc and carefully dried to evaporate the residual solvent. Standard kanamycin disc (30µg/disc) was used as the positive control in the experiment.

**Cytotoxicity by brine shrimp lethality bioassay:** In brine shrimp lethality bioassay DMSO solutions of the plant extracts were applied against Artemia salina in a 1-day *in vivo* assay, the experimental details of which could be found elsewhere [6, 7]. For the experiment, 4mg of each of the Kupchan fraction was dissolved in DMSO and solutions of varying concentrations (200, 100, 50, 25, 12.50, 6.25, 3.125, 1,563, 0.781, 0.390 µg/ml) were obtained by serial dilution technique.

The median lethal concentration ($LC_{50}$) of the test samples after 24 hours was obtained by a plot of percentage of the shrimp killed against the logarithm of the sample concentration.

**TABLE1: ANTIMICROBIAL ACTIVITY OF TEST SAMPLE OF *TINOSPORA CORDIFOLIA***

| Test microorganisms | Zone of Inhibition (in mm) | |
|---|---|---|
| ***Gram positive bacteria*** | **CTSF (400µg/ disc)** | **Kanamycin (30µg/ disc)** |
| *Bacillus cereus* | 7 | 33 |
| *Bacillus megaterium* | 8 | 33 |
| *Bacillus subtilis* | 10 | 34 |
| *Staphylococcus aureus* | 9 | 35 |
| *Sarcina lutea* | 8 | 33 |
| **Gram negative bacteria** | | |
| *Escherichia coli* | 10 | 32 |
| *Pseudomonas aeruginosa* | 8 | 33 |
| *Salmonella paratyphi* | 8 | 34 |
| *Salmonella typhi* | 9 | 35 |
| *Shigella boydii* | 8 | 33 |
| *Shigella dysenteriae* | 9 | 33 |
| *Vibrio mimicus* | 10 | 34 |
| *Vibrio parahemolyticus* | 8 | 32 |
| **Fungi** | | |
| *Candida albicans* | 8 | 35 |
| *Aspergillus niger* | 9 | 33 |
| *Sacharomyces cerevacae* | 8 | 33 |

**CTSF**: Carbon tetrachloride soluble fractions of the methanolic extract of *Tinospora cordifolia*

**Statistical analysis:** For each of the extractives, three samples were prepared for each of the bioassay. The zone of inhibition and $LC_{50}$ were calculated as mean (n=3) for the antimicrobial screening and brine shirmp lethality bioassay, respectively.

**RESULTS AND DISCUSSION:** In the antimicrobial screening, only the carbontetrachloride fractions of methanolic extract were found to be sensitive against almost all microorganisms. The carbontetrachloride partitionate at a concentration of 400 μg/disc, showed moderate to mild activity against the entire tested microorganism. Table 1 shows the antimicrobial activity of carbontetrachloride soluble fraction of Tinospora cordifolia of methanolic extract and kanamycin standard. The growth of *E. coli*, *Bacillus subtilis*, *Vibrio mimicus* were moderately inhibited with the zone of inhibition 10 mm, while, it showed low inhibitory activity against *S. aureus* (9mm), *S. lutea* (8 mm), *B. cereus* (7mm), *B. megaterium* (8 mm), *S. paratyphi* (8mm), *S. typhi* (9mm), *S. boydii* (8 mm) and the remainings. In case of fungi, the growth of *Aspergillus niger* mildly inhibited (9mm).

During the brine shrimp lethality bioassay, the lethality of the Pet ether (PE), Carbontetrachloride (CT), Chloroform (CF) and aqueous soluble fractions (AQF) of the methanolic extract to brine shrimp was determined on A. salina. Table 2 shows the results of the brine shrimp lethality testing after the 24 hours of exposure to the samples and the positive control, vincristine sulphate (VS). The $LC_{50}$ obtained from the best-fit line lope were found to be 1.918, 0.402, 0.691, 2.246, 0.323μg/ml for pet ether, Carbontetrachloride, Chloroform and aqueous soluble fractions of the methanolic extract and vincristine sulfate, respectively.

**TABLE 2: $LC_{50}$ DATA OF *T. CORDIFOLIA* EXTRACTIVES AND VINCRISTINE SULFATE**

| Samples | $LC_{50}$(μg/ml |
|---|---|
| VS | 0.323 |
| PE | 1.918 |
| CT | 0.402 |
| CF | 0.691 |
| AQF | 2.246 |

The values of $LC_{50}$ are expressed as mean (n=3). VS: vincristine sulphate (Std); VS: Vincristine sulphate (Std.), PE: Pet ether fraction of methanolic extract, CT: Carbontetrachloride soluble fraction of the methanolic extract, CF: Chloroform soluble fractions of methanolic extract, AQF: Aqueous fraction of the methanol.

**CONCLUSION:** In conclusion, our observation confirm that Carbontetrachloride extracts are better than that of Petroleum ether and Chloroform extracts of Tinospora cordifolia in respect to their antimicrobial activity and cytotoxicity and makes it a promising indigenous drug.

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