- Short communication

# Antimicrobial and Cytotoxic Activities of *Dillenia pentagyna*

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The plants of Dilleniaceae family grow well in hilly areas of Bangladesh such as Chittagong, Modhupur, Mymensing, Tangail, Chittagong, Rangpur, Sylhet and Dhaka Districts.[1,2] A wide range of pharmacological and biological activities was exhibited by the secondary metabolites isolated from plants belonging to Dilleniaceae.[3] Among them terpenoids and flavonoids were the most well known, though a large amount of alkaloid was also searched.[3,4] Betulinic acid was isolated from *Dillenia indica* & *D. return*,[4] which exhibited high antitumor activity.[5]

Two new compounds dihydro-isorhamnetin from the stem bark and dillenetin from the pericarp of *Dillenia indica* have been isolated.[4] Rhammentin 3-glucoside also isolated from the *Dillenia pentagyna.*[3] Two new flavonoid glycosides, naringenin 7-galactosyl and dihedral quercetin 5-glactoside were isolated from *D. pentagyna* have been found to exhibit cytotoxic and lymphocytic activity.[6] A new diterpene, dipoloic acid isolated from the stem of *Dillenia pentagyna* exhibited cytotoxic activity.[6]

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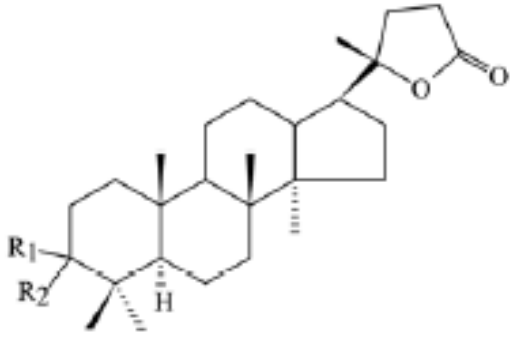
The stem bark of *D. pentagyna* was collected from the Madhupur Jungle of Tangail, Bangladesh. The sun-dried stem bark was ground mechanically and extracted in a Soxhlet apparatus successively with petroleum ether, ethyl acetate and methanol. The extracts were then concentrated in *vacuo* using a Buchi Rotary Evaporator. The EtOAc extract was then fractionated by vacuum liquid chromatography (VLC) over silica gel. Pure compounds were then isolated and purified from different fractions by preparative thin layer chromatography.

The antibacterial and antifungal activity of the crude extracts as well as for the isolated pure compounds were determined in vitro by disc diffusion technique.[7] Fifteen bacterial strains, which included seven gram-positive and eight gram-negative organisms, and nine fungi were collected from the Department of Microbology and Institute of Nutrition and Food Sciences, University of Dhaka. Nutrient agar media was used for culture of bacteria and potato dextrose agar media was used for the culture of fungi. Crude extracts were dissolved respectively in $CHCl_3$ having 3 mg of extract in each 30 ml of solvent. Selected Fractions were dissolved in the same way. Isolated compounds were dissolved with $CHCl_3$.[8] The sreile Matricel (BBL, cocksville USA) filter paper discs were impregnated with known amounts of the test substances and dried.

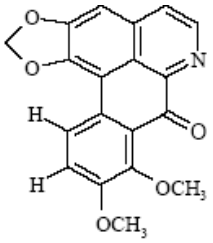
Standard ampicillin disc (l0µg/disc) and disc on which $CHCl_3$ was adsorbed and dried (blank disc) were used as positive and negativre controls, respectively. Standard grisofulvin disc (25 mg/disc) used incase of fungi.

The disc were then placed in petridises (120 mm in diameter) containing Mueller- Hinton agar media seeded with the test organisms using sterile cotton swabs. The plates were then incubated at $37^0$C for 24 hours. The antimicrobial activities were measured by zone of inhibition expressed in mm. All experiments were carried out in triplicate and the mean of the readings were recorded. The cytotoxic activities were performed by Brine shrimp lethality test.[9]

All the gram-positive strains showed sensitivity to EtOAc extract (3mg/disc), but promising activity found against *Bacillus subtilis, Bacillus megaterium* and *Staphylococcus β-haemolyticus* (Table 1). The petroleum ether extract demonstrated promising sensitivity against *Bacillus subtilis, Bacillus megaterium* and *Staphylococcus β-haemolyticus*. The methanol extract did not show any sensitivity. Most of the gram-positive bacterial strains exhibited sensitivity (Table 1) against different fractions of EtOAc extract (F-9, F-10, F-11. etc) except *Sarcina lutea*. But fractions 13, 14 to 15 showed promising activity against *Bacillus subtilis, Bacillus cereus* and *Bacillus megaterium.* All the gram-positive bacterial strains exhibited promising sensitivity (Table 1) against pure compounds DP-1, DP-2, DP-3 and DP-4.




DP-1: Cabralealactone




DP-2: Oxocrebanine

Most of the gram-negative bacterial strains demonstrated promising sensitivity (Table 2) against EtOAc extract. But Pet. Ether extract showed less activity to some of the gram-negative strains. Among the gram-negative organisms, *S. dysenteriae type-1* and *V. Cholerae.* exhibited promising sensitivity towards fraction F-5 to F-22 of EtOAc extracts. The early fractions showed mild sensitivity against all the gram-negative organisms. Pure compound DP-2 showed promising activity against *S. dysenteriae type-1* and *V. Cholerae.* DP-3 showed promising activity against *S. dysenteriae type-I ,S. basic, Vibrio cholerae* and *S. flexneri type-1*.

Studies on the antifungal activities showed that EtOAc extract, its fraction F-15 to F-18 and Grisofulvin have shown promising zone of inhibition against the fungi except *Candida albicans* and *Candida krusei* (Table 3). MeOH extract had no activity.

It was found from the result of the brine shrimp lethality test (Table 4) that the crude EtOAc extract exhibited toxicity towards brine shrimp. Test samples showed different mortality rate at different concentrations. The mortality rate of brine shrimp was found to be increased with the increase of the concentration for each sample. The percent mortality of the brine shrimp nauplii was calculated for every concentration for each sample. A plot of log concentration of the sample versus percent of mortality showed an approximate linear correlation between them. The $LC_{50}$ value of the crude ethyl acetate extract was 19.05 µg/ml and for the fractions 13 and 14 were 19.95 and 25.12 µg/ml respectively. Because of the shortage of PE and methanol extract these were not included in brine shrimp lethality test. From the above study, it may be concluded that this plant may be a very good source of natural medicine.

**Table 1. Antibacterial activitity of different extracts and compounds of *Dillenia pentagyna* against gram-positive bacteria**

| | | *Bacillus cereus* | *Bacillus subtilis* | *Bacillus polymyxa* | *Bacillus megaterium* | *Sarcina lutea* | *Staphylococcus aureus* | *Staphylococcus β-haemolyticus* |
|---|---|---|---|---|---|---|---|---|
| Zone of inhibition after 24 hours incubation (mm ± SD) | Pet. Ether extract (3mg/disc) | 14 ± 0.4 | 16 ± 0.6 | 12 ± 0.2 | 18 ± 0.5 | 13 ± 0.3 | 14 ± 0.5 | 16 ± 0.4 |
| | EtOAc extract (3 mg/disc) | 10 ± 0.6 | 12 ± 0.5 | - | 13 ± 0.8 | - | 10 ± 0.5 | 14 ± 0.3 |
| | MeOH extract (3 mg/disc) | - | - | - | - | - | - | - |
| | F-5 | - | - | - | - | - | - | - |
| | F-6 | - | - | - | - | - | - | - |
| | F-7 | - | - | - | 8 ± 0.5 | - | - | 8 ± 0.8 |
| | F-8 | - | 8 ± 0.4 | - | 8 ± 0.7 | - | 8 ± 0.3 | 12 ± 0.5 |
| | F-9 | 8 ± 0.3 | 9 ± 0.6 | - | 9 ± 0.4 | - | 8 ± 0.7 | 10 ± 0.6 |
| | F-10 | 8 ± 0.5 | 8 ± 0.4 | - | 10 ± 0.5 | 8 ± 0.6 | 9 ± 0.3 | 11 ± 0.4 |
| | F-11 | 8 ± 0.6 | 10 ± 0.4 | 8 ± 0.5 | 10 ± 0.8 | 8 ±0.5 | 9 ± 0.4 | 12 ± 0.7 |
| | F-12 | 10 ± 0.7 | 11 ± 0.3 | 8 ± 0.5 | 13 ± 0.4 | 9 ± 0.7 | 10 ± 0.8 | 10 ± 0.6 |
| | F-13 | 13 ± 0.4 | 14 ± 0.6 | 9 ± 0.3 | 17 ± 0.8 | 8 ± 0.4 | 9 ± 0.5 | 13 ± 0.5 |
| | F-14 | 13 ± 0.8 | 13 ± 0.5 | 10 ± 0.4 | 16 ± 0.7 | 10 ± 0.5 | 10 ± 0.4 | 14 ± 0.3 |
| | F-15 | 12 ± 0.6 | 12 ± 0.7 | 8 ± 0.5 | 12 ± 0.7 | 9 ± 0.5 | 9 ± 0.6 | 13 ± 0.8 |
| | F-16 | 10 ± 0.9 | 10 ± 0.5 | 8 ± 0.5 | 12 ± 0.6 | 8 ± 0.4 | 8 ± 0.8 | 10 ± 0.5 |
| | F-17 | 8 ± 0.5 | 9 ± 0.4 | - | 11 ± 0.7 | 8 ± 0.4 | 8 ± 0.8 | 9 ± 0.4 |
| | F-18 | 8 ± 0.7 | 8 ± 0.4 | - | 10 ± 0.5 | 9 ± 0.7 | 8 ± 0.5 | 8 ± 0.6 |
| | F-19 | - | 8 ± 0.6 | - | 8 ± 0.8 | - | - | 8 ± 0.4 |
| | F-20 | - | - | - | 8 ± 0.5 | - | - | 8 ± 0.7 |
| | F-21 | - | - | - | 8 ± 0.7 | - | - | - |
| | F-22 | - | - | - | - | - | - | - |
| | DP-1 | 9 ± 0.5 | 13 ± 0.6 | 8 ± 0.4 | 15 ± 0.9 | 8 ± 0.6 | 9 ± 0.4 | 12 ± 0.8 |
| | DP-2 | 8 ± 0.6 | 14 ± 0.7 | 9 ± 0.5 | 16 ± 0.8 | 9 ± 0.5 | 10 ± 0.7 | 11 ± 0.6 |
| | DP-3 | 11 ± 0.3 | 15 ± 0.8 | 10 ± 0.5 | 16 ± 0.5 | 10 ± 0.8 | 12 ± 0.9 | 14 ± 0.5 |
| | DP-4 | 10 ± 0.5 | 14 ± 0.6 | 9 ± 0.4 | 17 ± 0.8 | 9 ± 0.5 | 11 ± 0.8 | 13 ± 0.6 |
| | Ampicillin l0 µg/disc | 16 ± 0.8 | - | 10 ± 0.6 | 23 ± 0.9 | 15 ± 0.7 | 22 ± 0.5 | 24 ± 0.9 |

" - " = Indicates no Zone of inhibition.
F=Fraction, DP-1=Oxocrebanine, DP-2 = Cabralealactone, DP-3 =Unidentified compound, DP-4 = Unidentified compound

**Table 2. Antibacterial activitity of different extracts and compounds of *Dillenia pentagyna* against gram-negative bacteria**

| | | *S. soni* | *S. dysenteriae type-1* | *V. mimicus* | *S. basic* | *P. aeruginosa* | *V. Cholerae* | *S. flexneiy type-1* | *S. boydii* |
|---|---|---|---|---|---|---|---|---|---|
| Zone of inhibition after 24 hours incubation (mm ± SD) | Pet. Ether extract (3 mg/disc) | 11 ± 0.6 | 16 ± 0.8 | 12 ± 0.5 | 12 ± 0.4 | - | 13 ± 0.5 | 12 ± 0.6 | 11 ± 0.6 |
| | EtOAc extract (3 mg/disc) | 17 ± 0.4 | 23 ± 0.7 | 13 ± 0.5 | 18 ± 0.8 | 10 ± 0.5 | 20 ± 0.9 | 19 ± 0.7 | 17 ± 0.4 |
| | MeOH extract (3 mg/disc) | - | - | - | - | - | - | - | - |
| | F-5 | - | 10 ± 0.5 | - | 8 ± 0.7 | - | 8 ± 0.5 | ± 0.6 | 8 ± 0.5 |
| | F-6 | 8 ± 0.5 | 10 ± 0.7 | - | 8 ± 0.5 | - | 8 ± 0.4 | 8 ± 0.6 | 8 ± 0.8 |
| | F-7 | 8 ± 0.6 | 9 ± 0.4 | - | 8 ± 0.8 | - | 9 ± 0.7 | 9 ± 0.4 | 8 ± 0.5 |
| | F-8 | 9 ± 0.8 | 11 ± 0.7 | - | 9 ± 0.5 | - | 9 ± 0.4 | 10 ± 0.6 | 9 ± 0.7 |
| | F-9 | 9 ± 0.6 | 11 ± 0.9 | 8 ± 0.5 | 9 ± 0.6 | - | 10 ± 0.7 | 12 ± 0.8 | 9 ± 0.4 |
| | F-10 | 10 ± 0.7 | 13 ± 0.5 | 8 ± 0.7 | 10 ± 0.6 | - | 12 ± 0.5 | 14 ± 0.9 | 10 ± 0.5 |
| | F-11 | 11 ± 0.5 | 16 ± 0.8 | 8 ± 0.4 | 12 ± 0.8 | 8 ± 0.5 | 14 ± 0.9 | 14 ± 0.5 | 11 ±0.8 |
| | F-12 | 12 ± 0.4 | 18 ± 0.6 | 9 ± 0.5 | 14 ± 0.7 | 8 ± 0.7 | 15 ± 0.6 | 16 ± 0.8 | 11 ± 0.6 |
| | F-13 | 14 ± 0.8 | 22 ±0.7 | 10 ± 0.4 | 16 ± 0.5 | 9 ± 0.8 | 18 ± 0.7 | 18 ± 0.5 | 16 ± 0.4 |
| | F-14 | 16 ± 0.6 | 23 ± 0.9 | 10 ± 0.5 | 17 ± 0.7 | 9 ± 0.5 | 19 ± 0.6 | 17 ± 0.7 | 15 ± 0.8 |
| | F-15 | 13 ± 0.5 | 20 ± 0.8 | 8 ± 0.4 | 14 ± 0.6 | 8 ± 0.4 | 16 ± 0.4 | 15 ± 0.6 | 12 ± 0.5 |
| | F-16 | 10 ± 0.3 | 16 ± 0.5 | 8 ± 0.6 | 13 ± 0.8 | 8 ± 0.6 | 13 ± 0.3 | 13 ± 0.4 | 10 ± 0.6 |

**Table 2 (contd.)**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| F-17 | 9 ± 0.7 | 14 ± 0.6 | 8 ± 0.3 | 11 ± 0.5 | 8 ± 0.8 | 14 ± 0.9 | 12 ± 0.5 | 9 ± 0.7 |
| F-18 | 9 ± 0.5 | 10 ± 0.4 | - | 9 ± 0.4 | - | 10 ± 0.5 | 12 ± 0.3 | 9 ± 0.4 |
| F-19 | 8 ± 0.4 | 10 ± 0.3 | - | 9 ± 0.6 | - | 9 ± 0.7 | 11 ± 0.6 | 8 ± 0.5 |
| F-20 | 8 ± 0.6 | 9 ± 0.5 | - | 8 ± 0.7 | - | 9 ± 0.6 | 11 ± 0.8 | 8 ± 0.5 |
| F-21 | - | 9 ± 0.6 | - | 8 ± 0.4 | - | 9 ± 0.4 | 11 ± 0.5 | 8 ± 0.8 |
| F-22 | - | 9 ± 0.8 | - | 8 ± 0.5 | - | 8 ± 0.3 | 10 ± 0.7 | - |
| DP-1 | 14 ± 0.8 | 15 ± 0.5 | 9 ± 0.5 | 15 ± 0.7 | 8 ± 0.4 | 16 ± 0.6 | 14 ± 0.8 | 13 ± 0.6 |
| DP-2 | 13 ± 0.5 | 17 ± 0.8 | 8 ± 0.4 | 16 ± 0.9 | - | 17 ± 0.8 | 13 ± 0.6 | 11 ± 0.5 |
| DP-3 | 15 ± 0.7 | 19 ± 0.6 | 11 ± 0.7 | 17 ± 0.8 | 10 ± 0.5 | 18 ± 0.9 | 17 ± 0.7 | 15 ± 0.8 |
| DP-4 | 15 ± 0.9 | 21 ± 0.7 | 9 ± 0.8 | 16 ± 0.6 | 8 ± 0.6 | 19 ± 0.7 | 15 ± 0.9 | 14 ± 0.4 |
| Ampicillin 0µg/disc | - | - | - | - | - | - | - | 35 ± 0.7 |

"- " = Indicates no Zone of inhibition, F = Fraction

*S. sonii = Shigella soni, S. dysenteriae type-1 = Shigella dysenteriae type-1, V. mimicus= Vibrio mimicus, S. bagdic = Shigella basic, P. aeruginosa = Pseudomonas aeruginosa, V. cholerae= Vibrio cholerae, S. flexneri-type I= Shigella flexneri-type I, S. boydii= Shigella boydii.*

**Table 3. Antifungal activities of the crude extracts and isolated compounds of *Dillenia pentagyna***

| | | *A. fumigatus* | *C. albicans* | R. oryzae | *T. sp.* | *C. arriza* | *C. krusei* | *S. cerevisiae* | *A. niger* | R. oryzae |
|---|---|---|---|---|---|---|---|---|---|---|
| Zone of inhibition of compounds | EtOAc extract (3 mg/disc) | 11 ± 0.6 | - | 10 ± 0.8 | 17 ± 0.4 | 12 ± 0.6 | - | 16 ± 0.5 | 10 ± 0.7 | 13 ± 0.5 |
| | MeOH extract (3 mg/disc) | - | - | - | - | - | - | - | - | - |
| | Grisofulvin (25 mg/disc) | 12 ± 0.4 | 15 ± 0.6 | 18 ± 0.9 | 12 ± 0.7 | 16 ± 0.5 | 14 ± 0.6 | 16 ± 0.8 | 18 ± 0.5 | 13 ± 0.6 |
| | F-15 | 14 ± 0.7 | - | 12 ± 0.5 | 19 ± 0.8 | 16 ± 0.6 | - | 18 ± 0.9 | 14 ± 0.4 | 14 ± 0.8 |
| | F-16 | 16 ± 0.5 | - | 14 ± 0.4 | 20 ± 0.5 | 15 ± 0.8 | - | 20 ± 0.6 | 12 ± 0.4 | 16 ± 0.5 |
| | F-17 | 18 ± 0.9 | - | 14 ± 0.6 | 25 ± 0.9 | 12 ± 0.7 | - | 22 ± 0.8 | 16 ± 0.6 | 17 ± 0.4 |
| | F-18 | 16 ± 0.6 | - | 16 ± 0.4 | 22 ± 0.7 | - | - | 19 ± 0.8 | 12 ± 0.5 | 10 ± 0.6 |

*A. fumigatus = Aspergillus fumigatus, C. albicans = Candida albicans, R. oryzae = Rhizopus oryzae, T. sp.= Trichoderma sp., C. arriza = Candida arriza, C. krusei = Candida Krusei, S. cerevisiae = Saccharomyces cerevisiae, A. niger = Aspergillus niger, R. oryzae = Rhizopus oryzae.*

**Table 4. Results of Brine shrimp lethality test of crude extract and selected fractions of D*illenia pentagyna***

| Test sample | Gr. | Con µg/ml | Brine Shrimp in each vial | Death in each vial | | Average Deathing | % Mortality | Log conc | $LC_{50}$ µg/ml |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | | | | |
| Crude | A | 25 | 20 | 12 | 12 | 12 | 60 | 1.4 | |
| | B | 50 | 20 | 13 | 14 | 13.5 | 67.5 | 1.70 | |
| | C | 100 | 20 | 15 | 17 | 16 | 80 | 2.00 | 19.05 |
| | D | 200 | 20 | 18 | 20 | 19 | 95 | 2.30 | |
| | E | 400 | 20 | 20 | 20 | 20 | 100 | 2.60 | |
| | O | 20 | 20 | 0 | 0 | 0 | 0 | - | |
| Fr-13 | A | 25 | 20 | 10 | 11 | 10.5 | 52.5 | 1.40 | |
| | B | 50 | 20 | 13 | 14 | 13.5 | 67.5 | 1.70 | |
| | C | 100 | 20 | 15 | 16 | 15.5 | 77.5 | 2.00 | 19.95 |
| | D | 200 | 20 | 19 | 18 | 18.5 | 92.5 | 2.30 | |
| | E | 400 | 20 | 19 | 20 | 19.5 | 97.5 | 2.60 | |
| | O | 20 | 20 | 0 | 0 | 0 | 0 | - | |
| Fr-14 | A | 25 | 20 | 9 | 10 | 9.5 | 47.5 | 1.40 | |
| | B | 50 | 20 | 13 | 12 | 12.5 | 62.5 | 1.70 | |
| | C | 100 | 20 | 15 | 14 | 14.5 | 72.5 | 2.00 | 25.12 |
| | D | 200 | 20 | 17 | 18 | 17.5 | 87.5 | 2.30 | |
| | E | 400 | 20 | 18 | 20 | 19.0 | 95.0 | 2.60 | |
| | O | 20 | 20 | 0 | 0 | 0 | 0 | - | |

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