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In vitro Antibacterial Principles of Extracts and Two Flavonoids from *Clerodendrum indicum* Linn

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Abstract: Two flavonoidal compounds, pectolinarigenin (CA-2) and hispidulin (CA-3) isolated from the stem and root of the plant *Clerodendrum indicum.* The petroleum ether, chloroform and ethyl acetate extracts of the same portion of the plant were screened against twelve pathogenic bacteria for their antibacterial activities. The test materials found to be significant *in vitro* antibacterial activities against almost all gram positive and gram negative bacteria. The zones of inhibition produced by the test materials were found to be between 6 mm and 22 mm. The MIC values of the isolated compounds were also determined against four test organisms for CA-2 and two test organisms for CA-3. The values were found to be between 64 μg/ml and 128 μg/ml.

Key words: *In vitro*, antibacterial, extract, flavonoid, *Clerodendrum indicum*, verbenaceae

Introduction

Verbenaceae is a large plant family consisting of trees, shrub and herbs (Trease and Evan, 1983). Modern research carried on the Verbenaceous plants revealed that most of the plants belonging to this family are medicinally important as they contain biologically active compounds. *Clerodendrum indicum* Linn, locally known as Bamanhatti or Vamot in Bangladesh, belongs to the family Verbenaceae. it has considerable reputation for its medicinal values as traditional medicine. The root is considered useful in asthma, cough and scrofulous affections, the resin is employed in Syphilitic rheumatism and the juice of leaves is used with ghee as an application in hepatic eruptions and pemphigus (Kirtikar and Basu, 1994; Watt, 1972).

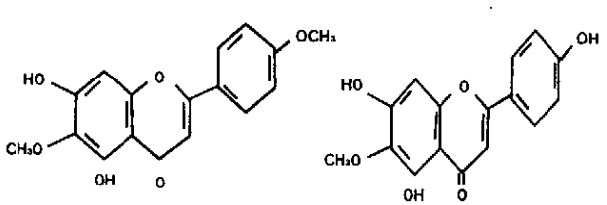
Although *C. indicum* is locally used for the above conditions, no antibacterial study of this plant has previously been reported. As a part of continuing search for novel antibacterial principles from the medicinal plants of Bangladesh, we studied *C. indicum* and herein report the result of *in vitro* antibacterial investigation.

Materials and Methods

Plant materials: Matured stem and root were collected from botanical garden, Rajshahi University, Rajshahi, Bangladesh and Sericulture Institute, Rajshahi, Bangladesh during the month of November-December, 1997. The plant was taxonomically identified by Professor A.T.M. Nadiruzzaman, Department of Botany, Rajshahi University, Bangladesh as well as the Bangladesh National Herbarium, Dhaka, Bangladesh where a voucher specimen is kept.

Extraction, isolation and characterization: The stem and root were dried in an oven at 45°C, crushed and then extracted separately in soxhlet apparatus using ethanol (95%) for 72 hrs at 70°C. The concentrated ethanolic extracts were diluted with distilled water and solvent-solvent partitioning were successfully carried out by Kupchan method (Grode *et al*., 1983) using petroleum ether, chloroform and ethyl acetate (Rahman, 1999). Each of the extract was concentrated at reduced pressure and appropriate temperature using rotary evaporator and thus ready for antibacterial screening.

The compound CA-2 was isolated from the petroleum ether and chloroform extract by column chromatography (Beckett and Stenlake, 1986) followed by TLC and PTLC (Egon, 1969). The compound CA-3 was isolated from chloroform extract by column chromatography (Beckett and Stenlake, 1986) followed by TLC and PTLC (Egon, 1969). These compounds were characterized on the basis of their UV, IR, NMR, MASS and NOE data and comparison with reported data (Hase *et al*., 1995). Then CA-2 and CA-3 were subjected to antibacterial screening.



Pectolinarigenin (CA-2) Hispidulin (CA-3)

Antibacterial Screening: Twelve pathogenic bacteria (six gram positive and six gram negative) were selected for the test and collected from the Department of Microbiology, Dhaka University, Dhaka, Bangladesh. Nutrient agar was used as bacteriological medium. The petroleum ether, chloroform and ethyl acetate extracts were dissolved separately in sufficient amount of methanol to get a concentration of 400 μg per 10 μl. Compound CA-2 and CA-3 were also dissolved separately in methanol in the same way to get a concentration of 200 μg per 10 μl. Then *in vitro* antibacterial activity of these samples were carried out by the standard disc diffusion method (Barry, 1980; Berghe and Vlietnck, 1991; Bauer *et al*., 1966; Rios *et al*., 1988) against selected test organisms. The diameter of zone of inhibition produced by the extracts and flavonoids

was then compared with those produced by the standard antibiotic (Kanamycin 30 µg/disc). The experiment was carried out in duplicate to minimize the error.

Minimum Inhibitory Concentration (MIC): The MIC value of the compound CA-2 was determined against two gram positive (*Bacillus subtilis* and *Sarcina lutea*) and two gram negative (*E. coli* and *Shigella dysenteriae*) bacteria. To determine the MIC value of CA-3, *Bacillus subtilis* and *Shigella dysenteriae* were taken as test organisms. The test was carried out by serial tube dilution technique (Reiner, 1982). Nutrient agar and nutrient broth were used as bacteriological media.

Results and Discussion

In antibacterial screening, it was found that all the three extracts showed a significant antibacterial activity. The isolated compounds (CA-2 and CA-3) also exhibited moderate activity against almost all test organisms (Table 1).

The zone of inhibition of petroleum ether extract (stern), chloroform extract (stem), chloroform extract (root) and ethyl acetate extract (root) were found to be 11-22 mm, 15-21 mm, 9-15 mm and 15-22 mm at a concentration of 400 µg/disc, respectively. The compound CA-2 and CA-3 produced zone of inhibition between 6 and 12 mm and 9 and 12 mm at a concentration of 200 pgldisc, respectively (Table 1).

The MIC values of CA-2 were 64 pglml against *B. subtilis* and *E. coli* and 128 µg/ml against *Samna lutea* and *Shigella dysenteriae.* For the compound CA-3, the MIC value was 128 µg/ml against *B. subtilis* and *Shigella dysenteriae* (Table 2).

From the experimental results it is evident that both the crude extracts and the flavonoidal compounds, CA-2 and CA-3, showed significant activity against the bacteria tested. Chloroform extract of stem displayed more activity than that of root against most of the test bacteria and in particular against *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus*, *Shigella shiga* and *Shigella dysenteriae.* It was also found that petroleum ether extract of stem was totally inactive against *Steptococcus-β-haemoliticus*, whereas others are moderate active. Ethyl acetate extract of root showed the highest activity against most of the test bacteria and in particular against *Shigella boydii*, *Shigella dysenteriae* and *Bacillus subtilis.* The extracts of root part exhibited more prominent antibacterial activity than that of stem part. The significant inhibitory activity of the extracts of root towards

Table 1: Antibacterial activities of extracts, CA-2, CA-3 and Kanamycin standard

Test organism	Diameter of zone of inhibition in mm						
	A	B	C	D	E	F	G
Gram positive:							
1. Bacillus subtilis	14	20	13	21	12	11	25
2. Bacillus megaterium	14	15	15	19	11	10	21
3. Bacillus cereus	22	19	11	20	12	10	27
4. Staphylococcus aureus	15	19	10	17	6	10	27
5. Streptococcus-β-*haemoliticus*	-	17	13	15	7	11	30
6. Sarcina lutes	12	16	12	18	11	9	22
Gram negative:							
1. Escherichia coli	19	15	12	19	7	9	20
2. Shigella dysenteriae	15	16	12	21	8	10	23
3. Shigella shiga	15	18	9	20	10	10	19
4. Shigella boydll	14	15	15	22	11	11	21
5. Shigella sonnei	11	21	14	17	7	10	22
6. Shigella flexneriae	16	19	9	18	9	12	19

A = Petroleum ether extract (stem), 400 µg/disk
B = Chloroform extract (stem) 400 pg/disk
C = Chloroform extract (root), 400 µg/disk
D = Ethyl acetate extract (root) 400 pg/disk
E = CA-2, 200 µg/disk
F = CA-3, 200 pg/disk
G = Standard Kanamycin, 30 pgidisk
(-) = No sensitivity

Table 2: The MIC values of the isolated compounds against test organisms

Sample	Minimum inhibitory concentration in µg/ml			
	B. subtilis	*S. lutes*	*E. coil*	*S. dysenteriae*
CA-2	64	128	64	128
CA-3	128	-	-	128

Shigella boydii, Shigella dysentariae, Shigella shiga, Bacillus subtilis and *Bacillus cereus* might indicate the traditional use of *Clerodendrum indicum* in different types of dysentery and diarrhoea.

The flavonoid, CA-2 showed more inhibitory activity against most of gram positive bacteria than the flavonoid, CA-3. On the other hand CA-3 exhibited more inhibitory activity against most of the gram negative bacteria than the flavonoid, CA-2. From the MIC values of CA-2 and CA-3 (Table 2), CA-2 was found to be more potent antibacterial compound against *Bacillus subtilis* and *E. coli* possessing less MIC value than CA-3 against *Bacillus subtilis*.

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