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Biological Activities of Extracts and two Flavonoids from *Oroxylum indicum* Vent. (Bignoniaceae)

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Abstract: The chromatographic examination of the ethyl acetate extract of a local plant ***Oroxylum indicum*** Vent. (Family: Bignoniaceae) has led to the isolation of two flavonoids: 2,5-dihydroxy-6,7-dimethoxy flavone (Compound 1) and 3, 7, 3′, 5′ -tetramethoxy-4′-hydroxy flavone (Compound 2). The ***in vitro*** anti microbial activity of crude petroleum ether, ethyl acetate and methanol extracts and compound 1 and 2 was screened against fourteen (five Gram-positive and nine Gram-negative) pathogenic bacteria and seven pathogenic fungi using the disk diffusion method. The zones of inhibition produced by the crude petroleum ether, ethyl acetate and methanol extracts and compound 1 and 2 against pathogenic bacteria were found between 8-16, 8-19, 0-8, 9-18 and 9-21 mm, respectively and against pathogenic fungi, 8-20, 8-18, 9-15, 9-20 and 7-18 mm, respectively. The crude petroleum ether and ethyl acetate extracts and compound 1 and 2 showed mild to moderate activity against all bacteria and fungi, whereas the methanol extract showed little activity against bacteria but moderate activity against fungi. The minimum inhibitory concentration (MIC) of the compound 1 and 2 were measured against ***Bacillus subtilis***, ***Staphylococcus aureus***, ***Escherichia coli*** and ***Shigella dysenteriae*** and the values were found to be between 64-128 μg/ml.

Key words: ***Oroxylum indicum***, Bignoniaceae, 2,5-dihydroxy-6,7-dimethoxy flavone, 3, 7, 3′, 5′-tetramethoxy-4′-hydroxy flavone, antibacterial and antifungal activity

Introduction

Plant have been playing as curative and therapeutic agents in preserving human health against diseases and decay since the beginning of man's life on earth. Bangladesh being a subtropical country is a good repository of plants that are being used in the preparation of local herbal medicine. Recent work in our laboratory on the chemistry of Bangladeshian medicinal plants have led to the isolation of various antibacterial, antifungal and cytotoxic principle (Rahman ***et al.***, 2000; Rashid ***et al.***, 2002). In continuation of our research, we selected a medicinal plant ***Oroxylum indicum*** Vent. (Family: Bignoniaceae) used in folk medicine as a cure of various diseases (Biswas and Ghosh, 1994). The root bark is used in fever, bronchitis, intestinal

worms, leucoderma, asthma, inflammation, anal troubles etc. The fruit and seeds are used as expectorant, purgative and bitter tonic (Kirtikar and Basu, 1996). In hindu medicine the root, bark, stem and leaf are prescribed for snake bite in diarrhea and dysenteries (Bhattacharya, 1980; Ghani, 1998). We here in, report the antibacterial and antifungal activities of extracts and two flavonoids 2,5-dihydroxy-6,7-dimethoxy flavone and 3, 7, 3´, 5´-tetramethoxy-4´-hydroxy flavone isolated first time from the ethyl acetate extract of root bark of the plant ***Oroxylum indicum***.

Materials and Methods

Collection of plant materials

The root barks of the plant ***Oroxylum indicum*** Vent. was collected from Gobindoganj, Gaibandha, Bangladesh during July-August, 2000. The plant was taxonomically identified by Professor A.T.M. Nadiruzzaman, Department of Botany, Rajshahi University, Bangladesh.

Extraction and isolation of Compound 1 and 2

The dried barks were crushed and extracted (850 gm) in soxhlet apparatus using petroleum ether in Phytochemistry Research Laboratory, Department of Pharmacy, Rajshahi University, Bangladesh. The residue was further extracted gradually with ethyl acetate and methanol. A portion of ethyl acetate extract was subjected to column chromatography on silica gel of 60-120-mesh size (Beckett and Stenlake, 1986). The column was first eluted with n-hexane and then n-hexane with increasing portions of ethyl acetate, then with ethyl acetate and finally with methanol which gave 29 fractions. Compound 1 was obtained from fraction 8-10 when subjected to PTLC (pet ether: ethyl acetate 3:1) while compound 2 was obtained from the fraction 11-16 using same solvent system. These two compounds were characterized as 2, 5-dihydroxy-6, 7-dimethoxy flavone and 3, 7, 3´, 5´-tetramethoxy-4´-hydroxy flavone by spectroscopic methods (by UV, IR and NMR, Uddin, 2001).

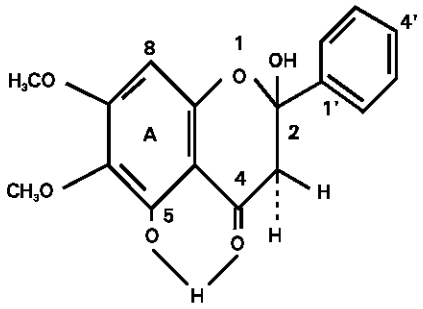

Fig. 1: 2, 5-dihydrosy-6, 7-dimethosy flavone (1)

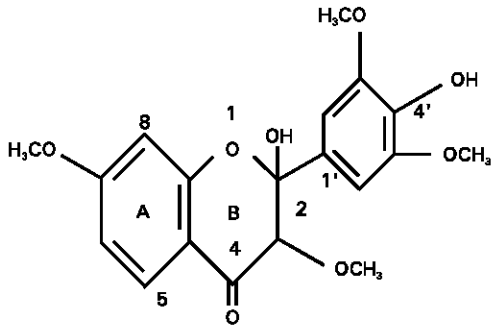

Fig. 2: 3, 7, 3', 5'-tetramethoxy-2hydroxy flavone (2)

Antimicrobial Screening

In vitro antimicrobial activity was performed with the crude extracts (petroleum ether, ethyl acetate and methanol) and compound 1 and 2 against fourteen pathogenic bacteria (5 gram-positive and 9 gram-negative) and seven pathogenic fungi that were collected from the Institute of Nutrition and food, University of Dhaka and ICDDRB. Nutrient agar and nutrient broth were used as bacteriological media and potato dextrose agar (PDA) was used for fungal growth.

In antibacterial screening, each sample was dissolved in methanol at a concentration of 200 µg/10 µl. The activity of these samples was compared with the standard kanamycin disc (K-30 µg/disc) by the standard disc diffusion method (Srivastava, 1984; Beur ***et al.***, 1996). Similarly antifungal screening was performed at a concentration of 300 µg/disc for each sample and the activity was compared with the standard Clotrimazole disc (K-30 µg/disc).

Results and Discussion

The results of antibacterial activity of the extracts and the isolated compounds are shown in the Table 1. The crude petroleum ether and ethyl acetate extracts and compound 1 and 2 showed mild to moderate activities against the bacteria while methanol extract showed little or no activities against some gram positive bacteria (***Bacillus subtilis***, ***Bacillus megaterium***, ***Staphylococcus aureus***, ***Sarcina lutea***, ***Streptococcus-β- hemolyticus***) and some gram negative

Table 1: *In vitro* antibacterial activity of the different extracts and pure compound 1 and 2 of *Oroxylum indicum*

	Diameter of the zone of inhibition (mm)					
Name of bacteria	Petroleum ether extract (200 µg/disc)	Ethyl acetate extract (200 µg/disc)	Methanol extract (200 µg/disc)	Compound 1 (200 µg/disc)	Compound 2 (200 µg/disc)	K_{30} (30 µg/disc)
Gram-positive						
Bacillus subtilis	16	13	0	13	16	39
Bacillus megaterium	14	12	0	12	13	20
Sarcina lutea	15	8	0	10	12	31
Staphylococcus aureus	16	19	0	18	21	39
Strepto-β-haemolytica	12	12	8	10	11	19
Gram-negative						
Escherichia cali	8	10	0	11	11	27
Shigella dysenteriae	10	10	0	10	9	25
Shigella sonnei	11	10	0	10	10	25
Shigella shiga	13	12	0	11	12	31
Shigella boydii	16	15	0	9	9	37
Shigella flexneriae	11	11	5	11	11	25
Pseudomonas aeruginosa	14	12	7	10	12	25
Salmonella typhi	10	12	5	10	9	13
Klebsiella species	10	10	0	12	12	24

NB: K_{30} indicates standard kanamycin

Table 2: *In vitro* antifungal activity of the different extracts and compound 1 and 2 of *Oroxylum indicum*

	Diameter of the zone of inhibition (mm)					
Name of fungi	Petroleum ether extract (300 µg/disc)	Ethyl acetate extract (300 µg/disc)	Methanol extract (300 µg/disc)	Compound 1 (300 µg/disc)	Compound 2 (300 µg/disc)	Standard Clotrimazole (30 µg/disc)
Aspergillus fumigatus	8	8	9	9	7	21
Aspergillus niger	15	14	13	16	13	23
Candida species	20	18	15	16	14	18
Hensinela Colifornica	14	10	9	7	8	14
Aspergillus flavus	16	15	10	20	18	20
Rhizopus aurizae	12	10	12	10	10	21
Rhizopus oligasporum	13	12	10	9	12	20

Table 3: The MIC value of the compound 1 and 2

	Minimum inhibitory concentration in µg/ml	
Bacteria	Compound 1	Compound 2
Bacillus subtilis	128	128
Staphylococcus aureus	128	128
Escherichia cali	128	128
Shigella dysenteriae	64	128

bacteria (***E. coli***, ***Shigella dysenteriae***, ***Shigella shiga***, ***Shigella boydii***, ***Shigella sonnei***, ***Shigella flexneriae***, ***Salmonella typhi***, ***Pseudomonas aeruginosa***, ***Klebsiella species***). Thus from the result it is evident that the petroleum ether extract showed moderate activity against ***Bacillus subtilis***, ***Bacillus megaterium***, ***Staphylococcus aureus***, ***Sarcina lutea***, ***Shigella boydii and Pseudomonas aeruginosa*** while the ethyl acetate extract showed moderate activity against ***Staphylococcus aureus*** and ***Shigella boydii***, compound 1 against ***Staphylococcus aureus*** and compound 2 against ***Bacillus subtilis***. All the compound and extracts showed mild activity against the other gram positive and gram negative bacteria.

The results of antifungal activity of crude extracts (petroleum ether, ethyl acetate and methanol) and compound 1 and 2 are given in the Table 2 that demonstrated that all the extract and compound showed strong activity against ***Aspergillus niger*** and ***Candida*** species and moderate activity against other species.

Since the compound 1 and 2 showed good activity against ***Bacillus subtilis***, ***Staphylococcus aureus***, ***E. coli*** and ***Shigella dysenteriae***, so the MIC of the compounds were carried out against these bacteria (Table 3).

From the antibacterial experimental results, it is evident that the crude extracts (petroleum ether and ethyl acetate) and the compound 1 and 2 showed significant antibacterial and antifungal activity but were less potent than that of standard kanamycin and Clotrimazole whereas methanol extract showed little activities. The results of this study further justify the use of this plant in the management of microbial infection.

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References

Beckett, A.H. and J.B. Stenlake, 1986. Chromatography. *In*: Practical pharmaceutical chemistry. Vol. 2, 3rd ed., Delhi, India, pp: 75-76.

Beur, A.W., W.M.M. Kibry, J.C. Sherris and M. Truck, 1996. Antibiotic susceptibility testing by a standard disc method. Am. J. Clin. Pathol., 44: 493-496.

Bhattacharya, S., 1980. Chironjib Bonaushodi, Ananda Publishing Company, Calcutta, 4: 242.

Biswas, K. and S.E. Ghosh, 1994. Bharater Bonoushodi. 2nd Ed. printed in India, 3: 858.

Ghani, A., 1998. Medicinal Plants of Bangladesh, Published by Asiatic Society of Bangladesh, Dhaka, Bangladesh, pp: 10-30.

Kirtikar, K.R. and B.D. Basu, 1996. Indian medicinal plants, 2nd Ed., 3: 1730.

Rahman, M.A.A., A.T.M.Z. Azam, J.A. Khanam and M.A. Gafur, 2000. ***In vitro*** cytostatic activity of a flavonoid, pectolinarigenin, isolated from ***Clerodendrum indicum Linn.*** on ehrlich ascite carcinoma cell to mice. J. Asiat. Sos. Bangladesh, Sci., 26: 289-291.

Rahman, M.A.A., A.T.M.Z. Azam and M.A. Gafur, 2000. ***In vitro*** antibacterial principles of two flavonoids and extracts from ***Clerodendrum indicum Linn.*** Pak. J. Biol. Sci., 3: 1769-71.

Rashid, M.H.O., M.A. Gafur, M.G. Sadik and M.A.A. Rahman, 2002. Antibacterial and cytotoxic activities of extracts and isolated compounds from ***Ipomoea turpethum.*** Pak. J. Biol. Sci., 5: 597-599.

Srivastava, O.P., 1984. Technique for evaluation of antimicrobial properties of natural product. In: The use of pharmacological technique for evaluation of natural product. Edited by Dewn B.N. Srinath, R., pp: 75.

Uddin, K., 2001. Chemical and antimicrobial investigation of ***Oroxylum indicum*** Vent. M. Sc. thesis, Department of Applied Chemistry and Chemical Technology, University of Rajshahi, Bangladesh.