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A Flavonone from Leaves of *Zanthoxylum budrunga*: its *In vitro* Antimicrobial Activity and Cytotoxic Evaluation

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The research work was conducted to investigate the antimicrobial and cytotoxic evaluation of a flavonone. The flavonone, 5-methoxy-7-hydroxy flavonone (1), has been isolated from the leaves extract of *Zanthoxylum budrunga* and its structure was established on the basis of structural evidence. This is the first report of its occurrence from the plant. The compound (1) showed significant antimicrobial activity against a number of pathogenic bacteria and fungi. The minimum inhibitory concentration (MIC) of the compound (1) was found to be 16μg ml^{-1} against *Staphylococcus aureus*. The compound showed prominent cytotoxic activity the LC_{50} and LC_{90} values of the compound (1) were found to be 16.24 and 56.56μg ml^{-1}, respectively.

Key words: *Z. budrunga*, flavonone, antimicrobial, cytotoxicity

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Introduction

The genus *Zanthoxylum* belongs to the family Rutaceae is comprised of about 11 species (Hooker, 1875 and Prain, 1963). *Zanthoxylum budrunga*, a member of this family is locally known as "Bajna" (Chopra *et al.*, 1956; Kirtikar and Basu, 1993) in Bangladesh. This plant is used in our country as folk medicine. The fruit juice of this plant is used in the treatment of heart diseases, bronchitis, asthma, piles, dysentery, cholera, rheumatism and also in the diseases of mouth, teeth and throat (Kirtikar and Basu, 1993). They also reported that the essential oil of leaves, cure cholera and bark, cure dysentery, cough, headache and vomiting.

Chemical Investigation (Thappa *et al.*, 1976; Benerjee *et al.*, 1989; Ruangrungsi *et al.*, 1981; Tirilline and Stoppini, 1994) on *Z. budrunga*, has led to the isolation of a new monoterpene triol, trihydroxy-p-menthane form its roots and a rutaecarpine, two alkaloids, arborine, diatamnine and four volatile compounds, β-phelalndrenr, hydroxy α-sanshool, pipertone, that β-pinrne from its fruits. In our previous paper, we reported the isolation of three terpenes from the bark of *Z. budrunga* and their antimicrobial investigation (Islam *et al.*, 2001a). Recently, we reported that the significant antimicrobial activity and cytotoxicity of the chloroform extract of leaves of this plant (Islam *et al.*, 2001b). Therefore, we were interested to isolate bioactive principles from the leaves of this plant.

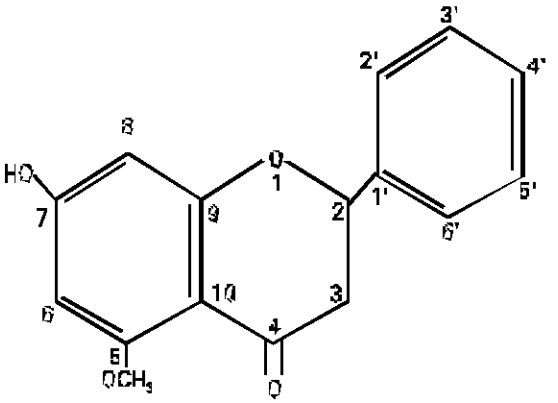
This communication deals with the isolation, structural elucidation, antimicrobial activity and cytotoxicity of a flavonone isolated from the chloroform extract ($CHCl_3$) of leaves of *Z. budrunga*.

Materials and Methods

Plant materials: Matured leaves of *Z. budrunga* were collected from a Hill in the district of Tangail of Bangladesh and the plant was identified by the Department of Botany, University of Rajshahi, where a voucher specimen has been deposited. The leaves were then dried in a oven at 40°C and pulverized into a fine powder, then stored in a airtight container.

Extraction, isolation and characterization: Air dried powdered materials of leaves (1 kg) were successively extracted with petroleum ether (40-60°C), chloroform (61°C) and methanol (64.6°C) in Soxhlet apparatus. The solvents were evaporated by a rotary evaporator under reduced pressure at 10°C temperature to afford a semisolid mass of petroleum ether ($C_2H_5O\ C_2H_5$), chloroform ($CHCl_3$) and methanol (CH_3OH) of 30.4, 20.3 and 8.2gm, respectively. From the antimicrobial activity and cytotoxicity of the different extracts of leaves of *Z. budrunga*, it was revealed that ($CHCl_3$) extract showed a significant activity (Islam *et al.*, 2001b). Sufficient amount of this extract was chromatographed on silica gel (G70-254 mesh, BDH Co. Ltd.) column packed in C_2H_5-O-C_2H_5 and then it was eluted successively with solvents by increasing polarity (Beckett and Stenlake, 1986). A total of 35 fractions, each of 100ml were collected. Fractions obtained with 50-65% ethyl acetate (CH_3-O-CO-C_2H_5) in petroleum ether (C_2H_5-O-C_2H_5) were bulked together and was subjected to preparative TLC (Egon and Stahl, 1969) using C_2H_5-O-C_2H_5, CH_3-O-CO-C_2H_5 (1:2) to afford (1) (0.45%). The R_f value over silica gel $60GF_{254}$ for compound (1) was 0.721. An infrared spectrophotometer (PERKIN ELMER, 1600), FTIR spectrophotometer, a nuclear magnetic resonance spectrophotometer (Vari VXR 500 MHZ) and a mass spectrophotometer (EIMS, 125 MHZ) were used in characterizing the compound (1).

Compound (1), crystals (CH_3OH), mp 125-129°C; IRVmax: 3550, 2850, 1680 and 1560 cm^{-1}; $EIMS_{m/z}$ (rel. int.%): 270 [M^+, 254], 250(54), 206(65), 170(100), 93(45), 77(16), 64(42), 47(34); ^1H NMR: δH 2.90 (1H, dd, J=16.4 and 2.5 H_α-3), 3.14 (1H, dd, J= 16.4 and 12.9 H_Z, H_β-3), 3.35 (s, 5-OCH_3), 5.57 (1H, dd, J=12.9 and 2.5 H_Z, H-2), 6.43 (1H, d, J=2.0 H_Z, H-6), 6.56 (1H, d, J=2.0 H_Z, H-8), 7.363 (br, t, J=7.1 H_Z, H4), 7.474 (t, J=7.1 H_Z, H-3, H-5), 7.610 (br, d, J=7.1 H_Z, H-2, H-6); ^{13}C NMR: δC 79.933 (C-2), 46.792 (C-3), 188.342 (C-4), 165.801 (C-5), 56.383 (5-OCH_3), 98.365 (C-6), 166.681 (C-7), 95.155 (C-8), 163.971 (C-9), 106.223(C-10), 140.524 (C-1`), 129.502 (C-2`, C-6`), 127.360 (C-3`, C-5`), 129.265 (C-4`).



Compound (1): 5-methoxy-7hydroxy flavonone

Antimicrobial screening: Tests for antimicrobial activity were carried out by standard disc diffusion method (Berghe and Vlietnck, 1991; Rios *et al.*, 1988). Twelve pathogenic bacteria (five gram positive and seven gram negative) and five pathogenic fungi were selected for the test and collected from the Department of Microbiology, Dhaka University, Dhaka, Bangladesh.

The isolated compound (1) was dissolved in sufficient volume of $CHCl_3$ to get a concentration of 200μg 10 μl^{-1} for antibacterial activity and 400μg 10 μl^{-1} for antifungal activity. The diameters of zone of inhibition produced by the compound were compared with those produced by the standard one, kanamycin 30μg $disc^{-1}$ for antibacterial and clotrimazole 30μg $disc^{-1}$ for antifungal activity.

Minimum Inhibitory Concentration (MIC): The MIC value of the isolated compound (1) was determined against a Gram positive bacteria, *Staphylococcus aureus*. The test was carried out by serial dilution technique (Reiner, 1982). Nutrient agar and nutrient broth were used as a bacteriological media.

Cytoto xic evaluation: Brine shrimp lethality bioassay is a recent development in the bioassay, for the bioactive compounds (Mclaughlin and Anderson, 1988 and Persoone, 1980). This bioassay is indicative of anticancer, antiviral, cytotoxicity and wide range of pharmacological activities of the compounds. However, we evaluated the cytotoxic effect of the compound (1) by this method. The compound (1) was dissolved in dimethylsulphoxide (DMSO) and five graded doses

5, 10, 20, 40, and 80μg ml^{-1}, respectively were used for 5ml sea water containing 10 brine shrimp nauplii in each group. The number of survivors were counted after 24 hours and LC_{50} and LC_{90} valueswere determined from the "log-dose response" curve (Goldstein *et al*., 1974).

Results and discussion

Chromatography over silica gel of $CHCl_3$ extracts of leaves of *Z. budrunga* afforded a pure compound (1) which was subsequently identified as 5-methoxy-7-hydroxy flavonone. The structure of this compound was elucidated by comparison of its melting point, IR, ^{1}HNMR, ^{13}CNMR and MS data with previously reported values (Hansel and Sauer, 1967).

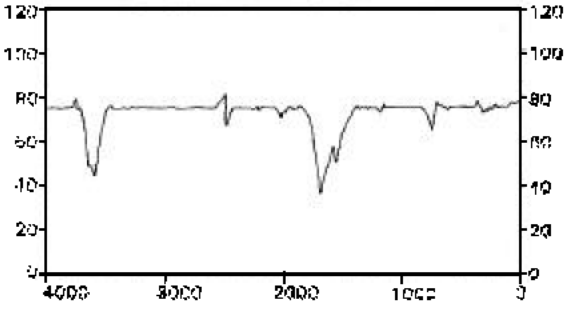


Fig. 1: IR Spectrum of compound (1)

Compound (1) was obtained as needles shaped crystal and had a melting point of 125-128^{0}C. It showed single spot on TLC plate when sprayed with vanillin-sulfuric acid by heating at 110^{0}C for 10 min. Its IR spectrum exhibited bands at 3550, 1680 and 1560 cm^{-1}, respectively (Fig.1), which could be attributed to hydroxyl group (OH), carbonyl group (CO) and aromatic substituent, respectively. The ^{1}HNMR spectrum (Fig. 2) exhibited a broad doublet at δ7.610 (J=7.1) and a triplet centered at δ7.474 (J=7.1) each integrated for two protons. These could be assigned to H-2`, H-6` and H-3`, H-5`, respectively. The ^{13}CNMR spectrum (Fig. 3) showed 16 carbons resonance and revealed the presence of one methyl (CH_3), one methylene (CH_2), eight methene and six quaternary carbons including four substituted with oxygen. Of the oxygenated quaternary carbons, one could be assigned to the carbonyl group at C-4 (δ188.342), one at C-9 (δ162.971) and another one to the methoxy (OCH_3) bearing carbon. Thus the fourth oxygenated quaternary carbon must contain a hydroxyl group (OH). The mass spectrum of the compound (Fig. 4) revealed the highest ion peak at m/z 270, which suggested the molecular weight 270 and molecular formula $C_{16}H_{14}O_4$. However, the compound is the first report from *Z. budrunga* belonging to Rutaceae family, previously reported from Piperaceae family (Hansel and Sauer, 1967).

The antimicrobial activity of the compound against pathogenic bacteria and fungi is presented in Table 1. The compound showed strong activity against *Staph. aureus*, moderate activity against *B. cereus*, *B. subtilis*, *E. coli* and *Sh. dysenteriae*, weak activity against *Streptococcus β-haemolyticus*, *Sh. flexneriae*, *Sh. Sonnei*, *Sh. boydii*, *Klebsiella*

Table 1: Antibacterial activities of compound (1) isolated form the leaves of *Z. budrunga*.

	Zone of inhibition (mm)	
Test Organism	Compound (1)	Standard Disc Kanamycin
Bacteria		
Gram positive		
Bacillus cereus	17	24
Bacillus subtilis	15	26
Bacillus megaterium	-	28
Staphylococcus aureus	22	27
Streptococcus β-haemolyticus	12	24
Gram negative		
Escherichia coli	14	27
Shigella dysenteriae	15	26
Shigella shiga	-	22
Shigella flexneriae	13	27
Shigella sonnei	12	23
Shigella boydii	12	25
Klebsiella species	13	19
Fungi		Clotrimazole
Aspergillus fumigatus	14	21
Hensinella californica	13	19
Rhizopus oligosporum	13	18
Schizosporum species	12	20
Rhizopus orizae	10	22

Compound (1) = 5-methoxy-7-hydroxy flavonone "-" = no sensitivity

Table 2: Minimum Inhibitory concentration (MIC) of compound (1) against *Staph. aureus*.

Dilutions	1	2	3	4	5	6	7	8	9	10	C_{MC}	C_{MI}	C_M
Diluted solution of the compound (μg ml^{-1})	512	256	128	64	32	16	8	4	2	1	512	0	0
Observation	NG	NG	NG	NG	NG	NG	G	G	G	G	NG	G	NG

C_{MC} =Medium +Compound; C_{MI}=Medium +Inoculum; C_M=Medium only
Compound (1) = 5-methoxy-7-hydroxy flavonone G = Growth; NG = No growth No. of cells: 10^7 ml^{-1}

Table 3: Brine shrimp lethality bioassay of compound (1) isolated form the leaves of *Z. budrunga*.

Test sample	Concentration (μgml^{-1})	LogC	% Mortality	LC_{50} (μg ml^{-1})	LC_{90} (μg ml^{-1})
Compound (1)	5	0.698	20		
	10	1.0	35		
	20	1.301	55	16.24	56.56
	40	1.602	75		
	80	1.903	100		

LC_{50} = 50% Mortality and LC_{90} = 90% Mortality

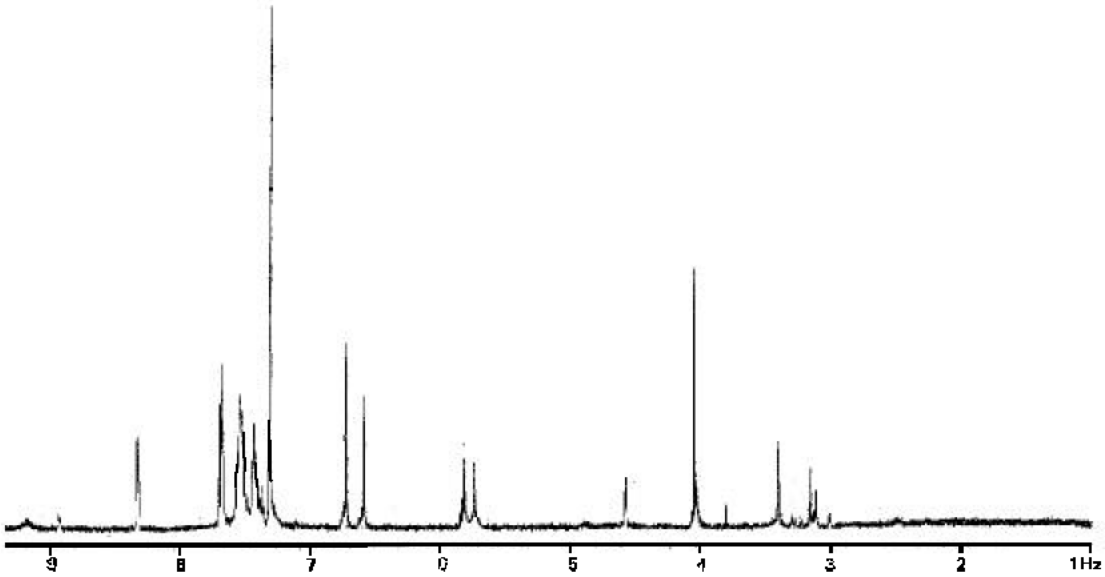


Fig. 2: ^{1}HNMR Spectrum of compound (1)

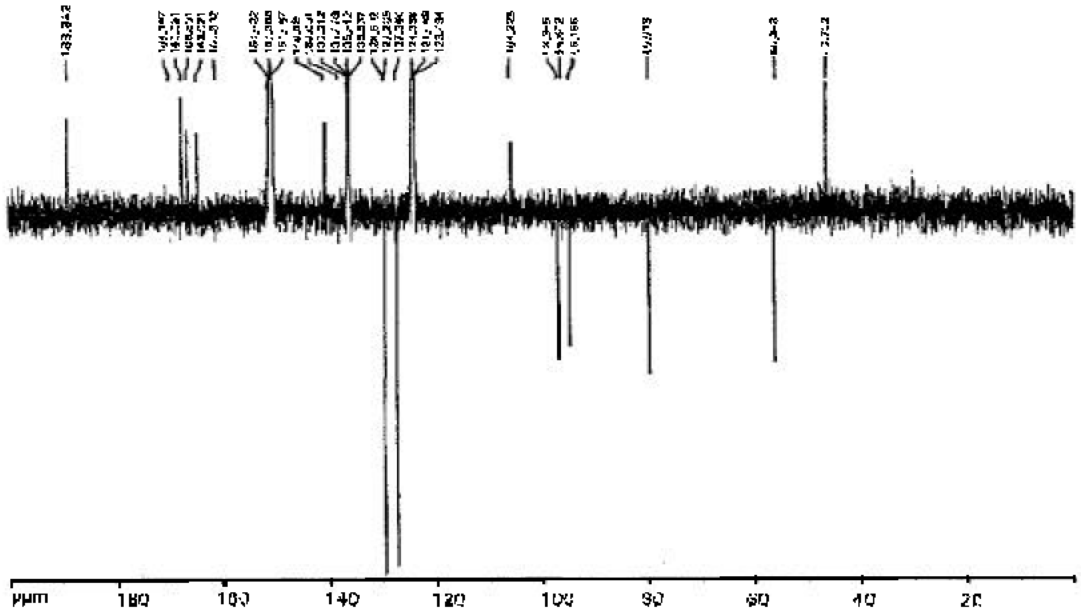


Fig. 3: ^{13}CNMR Spectrum of compound (1)

sp. and no activity against *B. megaterium* and *Sh. Shiga*. The compound also exhibited weak antifungal activity against all pathogenic fungi examined. As the compound was strongly active against *Staph. aureus*, the MIC value of the compound against the organism was checked and the results are presented in Table 2.

The cytotoxic activity of the compound was performed and the results are presented in Table 3. The 50% mortality (LC_{50}) and 90% mortality (LC_{90}) of the compound were found to be 16.24μg ml^{-1} and 56.56μg ml^{-1}, respectively. An approximate linear correlation was observed when logarithm of concentration versus percentage of mortality (Goldstein *et al.* 1974) was plotted on the graph paper and the results were obtained by extrapolation from the graph. Although there was no mortality in the control group, the test sample showed different mortality rate at different concentrations and was found to be increased with increasing concentration of the sample. It is evident that the test material was moderately

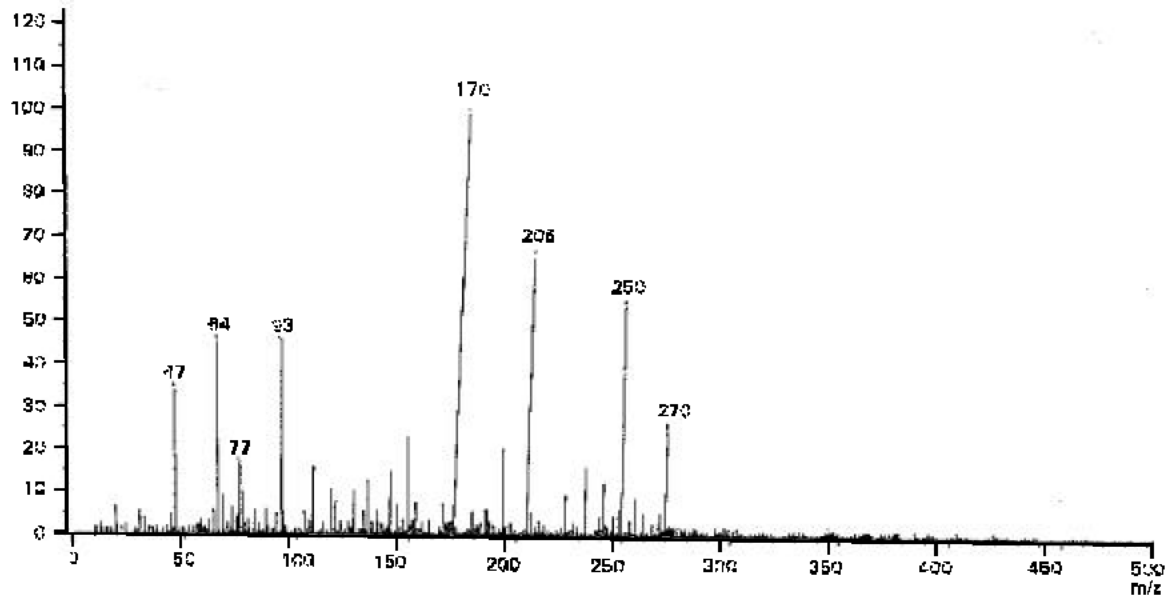


Fig. 4: Mass spectrum of compound (1)

lethal to brine shrimp nauplii. By disturbing the fundamental mechanisms concerned with cell growth, mitotic activity, differentiation and function, cytotoxic action of a drug is simply provided (Goodman *et al*., 1980). But at this time we are not clear about the exact mechanism of cytotoxic action of this drug. However, the better evaluation to the potential antimicrobial and cytotoxic effectiveness of the flavonone from the leaves of *Zanthoxylum budrunga* awaited further and specific studies.

Acknowledgments

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