http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (12): 2261-2266, 2006
ISSN 1028-8880

Isolation and Biological Activities of Chemical Constituents from the Stems of *Ipomoea turpethum*

[1,2]Md. Harun-Or-Rashid, [3]Nurul Karim, [1]M.A. Gafur, [1]Md. Golam Sadik, [1]A.S.M. Anisuzzaman, [4]Noaki Sugimoto and [5]A.T.M. Zafrul Azam
[1]Department of Pharmacy, Phytochemical Research Laboratory, Institute of Biological Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh
[2]Department of Health Chemistry, [3]Department of Biochemistry, Faculty of Pharmaceutical Sciences, Niigata University of Pharmacy and Applied Life Sciences, 265-1 Higashijima, Niigata 950-8603, Japan
[4]National Institute of Health Science, Tokyo, Japan
[5]Department of Chemistry, Faculty of Engineering, Gunma University, Japan

Abstract: Extensive chromatographic separation and purification of the organic solvent extracts of stem of *I. turpethum* afforded three compounds, *N-p*-coumaryl tyramine (EH4), daucosterol (CH1) and 2-hydroxybenzoic acid (CH2); first isolated from this plant. The structures of these compounds were determined by spectroscopic analysis, including ^{1}H- and ^{13}C-NMR, as well as by UV, IR and electrospray ionization mass spectrometry (ESI-MS). On screening against pathogenic bacteria and fungi; compounds CH2 and EH4 as well as extracts showed significant antibacterial and antifungal activity. The Minimum Inhibitory Concentration (MIC) against *Bacillus subtilis* and *Shigella dysenteriae* of compounds CH2 and EH4 were 64 μg mL^{-1}. On *in vivo* cytotoxicity studies against brine shrimp; compounds CH2 and EH4 showed LC_{50} (Leathal concentration for 50% mortality) values 54.03 and 43.41 μg mL^{-1}, respectively.

Key words: *I. turpethum*, Convolvulaceae, *N-p-*coumaryl tyramine, daucosterol, salicylic acid

INTRODUCTION

Now a days there is a growing interest in natural healing methods and particular efforts have been devoted to elucidate medicinal effects of natural agents in plant and the typical methods of plant extract preparations (Saieed *et al*., 2006). To make health care and medical facilities available to the people is now a major concern of the world. Due to the toxic and adverse effect of synthetic medicine being observed round the globe, herbal medicine has made a comeback to improve the fulfillment of our present and future health needs. Besides, herbal medicine can cope with the present economic conditions of our people who cannot afford to use the expensive medicine. There fore our work is primarily concerned with the isolation of bioactive compounds from medicinal plants of Bangladesh. In continuation of our investigation (Rahman *et al*., 2000) we choose a local plant Dudhkalmi having therapeutic value.

Dudhkalmi, scientific name *I. turpethum*, R. Br., synonym: *Operculina turpethum* Silva Manso Enum (Family-Convolvulaceae), is a large, climbing, shrub that is distributed in Bangladesh, India, Ceylon, tropical America, Mauritania, Philippines, tropical Africa and Australia. The leaves are very variable in shape; the flowers are tubular-campanulate, white, in few flowered cymes; the capsules globose with 4 or less, dull back, glabrous seeds. This plant produces milky juice and known to be effective against ascites, piles, snakebites, fever, etching, ulcer, bronchitis, muscle pain, constipation, anemia and jaundice in folk medicine (Kirtikar and Basu, 1994). Previous phytochemical studies with *I. turpethum* reported the isolation of β-sitosterol, betulin and lupeol (Nasar, 1982); some terpenoide cycloartenol, lanosta-5-ene and 24-methylene-δ 5-lanosterol (Sahabuddin, 1999). Broad-spectrum virus inhibitory activity of the aqueous leaves extract of this plant has also been documented (Khan, 1992). Alcoholic extracts of the fresh fruits showed

Corresponding Author: Nurul Karim, Department of Biochemistry, Faculty of Pharmaceutical Sciences, Niigata University of Pharmacy and Applied Life Sciences, 265-1 Higashijima, Niigata 956-8603, Japan Tel: +81-250-25-5274

antibacterial activities against *Micrococcus pyogens* var. *aurus* and *E. coli* (Welth of India, 1966). Recently there is a reputation of pharmacological active ingredients (Vitamin C, chlorogenic acid, caffeic acid, quercetin and reutin) from *I. batatas* (Guan *et al*., 2006) and a new alkaloid (N1,N10-ditigloylspermidine) from *I. nil* (Schimming *et al*., 2005) belongs to Convolvulaceae. So our vision is to find out bioactive compounds of therapeutic interest from *I. turpethum* of Convolvulaceae. We, herein, report the isolation, purification, structure determination and biological activities of N-p-coumaryl tyramine (EH4), daucosterol (CH1) and 2-hydroxybenzoic acid (CH2) for the first time from the stem extract of this plant.

MATERIALS AND METHODS

Plant materials: The stems of *I. turpethum* were collected from the Barendra region of Bangladesh and were taxonomically identified by Professor A.T.M. Naderuzzaman, Department of Botany, Rajshahi University, Bangladesh (Pub med cited specimen No. S4842).

Organisms and chemicals: Culture of bacteria and fungi and also shrimp eggs were collected from the institute of Nutrition and Food, University of Dhaka and International Centre for Diarrhoeal Disease Research of Bangladesh (ICDDRB). Kanamycin disc (K-30) and fuconazole disc (Fcz-100) from BBL and cookeville (USA); nutrient agar, nutrient broth and potato dextrose agar (PDA) media were purchased from Becton, dickinson and company (USA). Dimethyl sulphoxide (DMSO) from Wako pure chemical industries Ltd. (Japan). Precoated silica gel 60 F_{254} and silica gel (60-120 mesh size) from Merck (1966) (Darmstadt, Germany). All other chemicals and organic solvents were of the highest grade available.

General experimental procedures: Melting points were recorded on a Gallenkamp melting point apparatus and were uncorrected. UV spectra were recorded on a Beckman double beam spectrometer. IR spectra were obtained by a Perkin Elemer 1600 FTIR spectrometer. ^{1}H-(500 MHz) and ^{13}C-NMR (125 MHz) spectra were acquired on a JEOL JNM alpha spectrometer using TMS as internal standard. Electrospray ionization mass (ESI-MS) was recorded on a JEOL DX-300 spectrometer. The TLC (Thin layer chromatography) was carried out using precoated silica gel 60 F_{254} plates (Mercks) and detection was made by visualization under UV light (254 nm) and spraying with 0.1% vanillin sulphate spray reagent followed by heating.

Extraction and isolation: The air-dried and powdered stem (350 g × 3) was extracted by percolation (Rashwan, 2002) in methanol at 65°C. The methanol extract was evaporated to dryness under reduced pressure to give a solid residue (100 g). The residue was suspended with water (200 mL) and successively fractionated with petroleum ether, chloroform and ethyl acetate and evaporated to yield 30.5, 4.1 and 2.0 g, residue, respectively.

A portion (1.8 g) of ethyl acetate extract was chromatographed on silica gel (60-120 mesh) column (50×3 cm) using n-hexane-ethyl acetate mixtures of increasing polarities. The fractions eluted with n-hexane-ethyl acetate (1:1 and 1:2) were combined and subjected to preparative thin layer chromatography (PTLC) using n-hexane-ethyl acetate (1:5) (visualized under UV light at 254 nm) to afford EH4 (15.0 mg) (Fig. 1).

Similarly 4.0 g dried chloroform extract fractionated by column chromatography (CC) over silica gel (60-120 mesh size) eluting with a gradient flow of n-hexane: chloroform mixture with increasing the polarity. Solvent system n-hexane: ethyl acetate = 1:1 afforded CH2 (12 mg) and 100% chloroform afforded CH1 (20 mg) which are purified by PTLC (CH2: Chloroform: Methanol = 7:1. and CH1: Chloroform: Methanol = 4:1) (Fig. 1). The structures of the three compounds were determined conclusively by UV, IR, ^{1}H- and ^{13}C-NMR analysis.

Antibacterial activity: Antibacterial screening was performed with crude extracts (petrolieum ether, chloroform and ethyl acetate) and three isolated compounds (CH1, CH2 and EH4) against some pathogenic bacteria, viz., gram positive (*Bacillus subtilis*, *B. megaterium*, *Staphylcoccus aureus*, *Sarcina lutea*, *Stepto-β-hemolyticus* and *Sarcincina sarcinaceae*,) and gram negative (*E. coli*, *S. dystenteriae*, *S. Shiga*, *S. boydii*, *S. sonnei*, *S. flexneriae*, *Salmonella typhi*, *Klebsiella species* and *Psuedomonus aureginosa*). Nutrient agar and nutrient broth were used as bacteriological media. Crude extracts and compounds were dissolved in methanol at a concentration of 200 and 100 μg/10 μL, respectively. A disc with 10 μL methanol was a used as control. The antibacterial activity was compared with the standard kanamycin disc (k-30) by the standard disc diffusion method (Srivastava, 1984; Bauer *et al*., 1966; Masako *et al*., 2004). Inhibitory activity was measured (in mm) as the diameter of the observed inhibition zone.

MIC is defined as the lowest concentration that inhibits bacterial growth. The MIC of CH2 and EH4 was determined against gram positive *(Basillus subtilis*, *Surcina lutea*) and gram negative (*E. coli* and *Shigella dysenteriae*) bacteria (10^{7} cells/mL) by serial dilution technique (Reiner, 1982) in nutrient broth media.

Antifungal activity: Antifungal activity of compounds CH1, CH2 and EH4 as well as extracts towards some fungi were studied, viz., *Aspergillus flavus*, *A. niger*,

A. Versicolor and *Candida albicans*. Crude extracts and compounds were dissolved in DMSO at a concentration of 200 and 100 μg/10 μL, respectively. Disc with 10 μL DMSO as control and Fuconazole (100 μg/disc) as a standard fungicide were used. Antifungal activity was measured (in mm) as the diameter of inhibition zone in Potato Dextrose Agar (PDA) media by standard disc diffusion method (Bauer *et al*., 1966; Alam, 2004).

Cytotoxic activity: The *in vivo* cytotoxic activity of CH2 and EH4 was determined by the brine shrimp (*Artemia salina*) lethality bioassay and was compared with standard antibiotic ampicillin trihydrate (Mclaughlin and Andersion, 1988; Rashid *et al*., 2002). Briefly, Brine shrimp eggs were hatched for 48 h in saline water (3.8% NaCl) to mature as nauplii (larvae). Five milligram of each sample (Compounds CH2, EH4 and Ampicillin trihydrate) was dissolved in 1 mL DMSO. 5, 10, 20, 40 and 80 μL of test solution were taken in separate vials and 5 mL of saline water was added to each vial containing 10 nauplii. A control group was used containing 80 μL of DMSO and 10 nauplii in 5 mL saline water. After 24 h, the number of surviving nauplii in each vial was counted. The percentage of mortality of the nauplii was calculated and LC_{50} values were determined.

RESULTS AND DISCUSSION

A combination of partition chromatography (with petroleum ether, chloroform and ethyl acetate), CC and PTLC of hot methanol extract of stem of *I. turpethum* ware afforded compound CH1, CH2 and EH4 (Fig. 1).

Compound EH4 (N-p-coumaryl tyramine): Yellowish white, crystalline powder (MeOH), mp- 245°C- 247°C; UV (MeOH) λ_{max}: 306, 281 and 224 nm; IR ν_{max} (KBr) cm^{-1}: 1150, 1265, 1300, 1445(C-N stretching), 1590, 1625(- C = O), 2900, 3015(N-H stretching), 3120 and 3625; ^{1}H-NMR (500 MHz, CD_3OD) δ: 7.43 (1H, d, J = 15.6 Hz, H-3), 6.37 (1H, d, J = 15.6 Hz, H-2), 3.45 (1H, d, J = 7.4 Hz, H-CNH), 2.75 (2H, t, J = 7.4 Hz, H-CCH_2NH), 7.04 (2H, d, J = 8.2 Hz, H-2′), 6.71 (2H, d, J = 8.6 Hz, H-3′), 7.39 (2H, d, J = 8.5 Hz, H-2″) and 6.78 (2 H, d, J = 8.6 Hz, H-3″); ^{13}C-NMR (125 MHz, CD_3OD) δ: 141.8 (C-3), 118.5 (C-2), 169.2 (-C = O, C-1), 42.5 (C-NH), 35.8 (C-CH_2NH), 131.3 (C-1′), 130.7 (C-2′, 6′), 116.3 (C-3′,5′), 156.9 (C-4′), 127.8 (C-1″), 130.5 (C-2″, 6″), 116.7 (C-3″,5″) and 160.5(C-4″); ESI-MS m/z (% rel. int.): 283.15 [M^+]($C_{17}H_{17}NO_3$), 165.15, 164, 147(100%), 120, 119, 107, 91, 65, 51 and 45.71. The structure of EH4 was elucidated by comparing the reported data of N-p-coumaryl tyramine [3-(4-hydroxy-phenyl)-N-[2-(4-hydroxy-phenyl)-ethyl-acrylamide] (Zhao *et al*., 1992; Rahman *et al*., 1992; Spasova *et al*., 2005) first reported from *I. turpethum*.

Compound CH2 (2-Hydroxy-benzoic acid): White, needle shaped crystal ($CHCl_3$), mp. 156-157°C (Budavari *et al*., 1989); UV (MeOH) λ_{max}: 305.0, 233.0 and 212.0 nm; IR ν_{max} (KBr) cm^{-1}: 1625(-C = O), 1530, 3200, 3510, 1205, 1270, 2800. ^{1}H-NMR (500MHz, $CDCl_3$) δ: 7.94 (1H, dd, J = 6.92 and 7.996 Hz, H-6), 6.95 (1H, t, J = 7.996 Hz, H-5), 7.54 (1H, ddd, J = 7.996, 7.996 Hz, H-4), 7.02 (1H, d, J = 7.996 Hz, H-3) and 10.376 (1H, s, -COOH); ^{13}C-NMR (125MHz, $CDCl_3$) δ: 174 (-COOH), 111.3 (C-1), 162.0 (C-2), 117.8(C-3), 136.9(C-4), 119.6(C-5) and 130.9(C-6); ESI-MS: m/z (% rel. int.): 138.1 [M^+] ($C_7H_6O_3$), 120, 92 (100%), 64, 63, 53, 50 and 45.

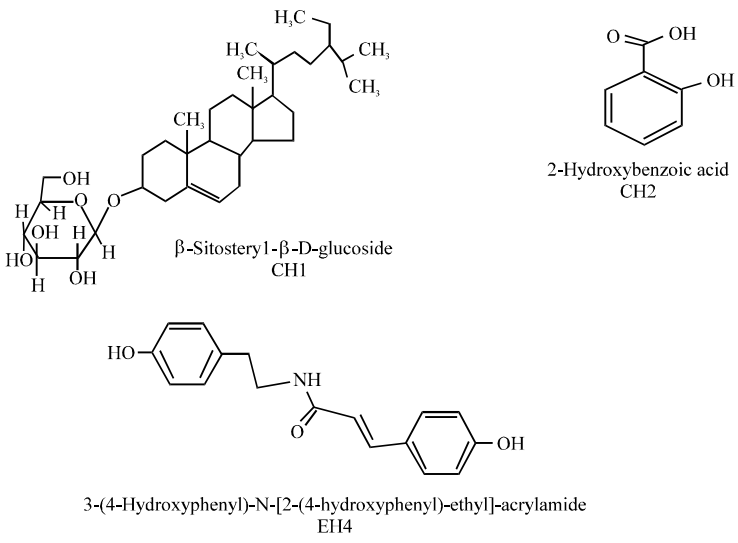

Fig. 1: Chemical structures of isolated compounds. The structures were determined as described in materials and methods

Table 1: *In vitro* antibacterial activities of extracts as well as of compounds CH1, CH2 and EH4

	Diameter of inhibition zone in mm						
Test organisms	I	II	III	CH1	CH2	EH4	K-30
Gram positive bacteria							
Bacillus subtilis	12	12	10	6	11	15	22
B. megaterium	8	9	8	0	13	11	20
Staphylcoccus aureus	15	13	19	7	12	16	22
Sarcina lutea	15	12	13	8	14	18	23
Stepto-β-hemolyticus	8	10	13	0	17	11	22
Sarcincina sarcinaceae	15	12	15	6	13	14	30
Gram negative bacteria							
E. coli	15	9	6	9	12	18	22
S. dysterteriae	10	9	10	0	9	12	21
S. Shiga	14	15	17	5	8	20	25
S. boydii	14	11	15	7	12	12	19
S. sonnei	14	14	12	10	14	13	23
S. flexneriae	10	10	11	9	12	18	19
Salmonella typhi	17	15	19	5	17	19	26
Klebsiella species	19	19	21	6	16	21	26
Psuedomonus aureginosa	14	12	12	7	15	15	21

[Petroleum ether (I), chloroform (II) and ethyl acetate (III) extracts (each 200 μg/disc); CH1, CH2 and EH4 compounds (each 100 μg/disc) and K-30 = Kanamycin (30 μg/disc)]

Table 2: The MIC (μg mL^{-1}) value of compound CH2 and EH4 against gram positive and gram negative bacteria

	Gram positive bacteria		Gram negative bacteria	
Sample	*Bacillus subtilis*	*Sarcina lutea*	*E. coli*	*S. dystenteriae*
CH2	64	64	128	64
EH4	64	64	128	64

The structure of CH2 was elucidated by comparing the reported data of salicylic acid (Springer-Verlag, 1983) first reported from this plant.

Compound CH1 (β-Sitosteryl-β-D-glucoside): Ash white, crystalline powder (MeOH), mp. 282-284°C; UV (MeOH) λ_{max}: 205.4 nm; IR ν_{max} (KBr) cm^{-1}: 3400, 1090, 2900, 1470 and 1375. ^{1}H-NMR (500MHz, C_5D_5N) δ: 1.724 (2H, m, H-1), 2.50 (1H, t, J = 11.76 Hz, H-2), 2.75 (1H,dd, J = 2.0, 2.5 Hz, H-2), 3.89 (1H, m, H-3), 1.41 (2H, m, H-4), 5.37 (1H, bs, H-6), 2.15 (2H, bd, J = 11.3 Hz, H-7), 1.94 (1H, bd, J = 11.3 Hz, H-8), 0.94 (1H, t like, H-9), 1.10 (2H, dt, 13.07 Hz, H-11), 1.85 (2H, m, H-12), 1.15 (1H, d, J = 13.02 Hz, H-14) 1.15 (1H, d, J = 13.02 Hz, H-14), 1.56 (2H, m, H-15), 1.27 (2H, m, H-16), 1.15 (1H, d J = 13.20 Hz, H-17), 0.68 (3H, s, H-18), 0.85 (3H, s, H-19), 1.95 (1H, m, H-20), 1.01 (3H, d, J = 7.0, H-21), 1.40 (2H, m, H-22), 1.40 (2H, m, H-22), 1.10 (2H, m, H-23), 1.09 (1H, m, H-24), 1.70 (1H, m, H-25), 0.91 (3H,d, J = 6.42 Hz, H-26), 0.87 (3H, d, J = 6.42 Hz, H-27), 1.30 (2H, m, H-28), 0.90 (3H, t, J = 6.5 Hz, H-29), 5.07 (1H, d, J = 8.2 Hz, H-1′), 4.09(1H, t, J = 8.3 Hz, H-2′), 4.31(1H, t, J = 8.07 Hz, H-3′), 4.32(1H, t, J = 8.07 Hz, H-4′), 4.0 (1H, m, H-5′), 4.45(1H, dd, J = 5.0, 11.67 Hz, H-6′), 4.59 (1H, dd, J = 2.50, 11.25 Hz, H-6′); ^{13}C-NMR (125 MHz, C_5D_5N) δ: 37.50(C-1), 32.081 (C-2), 78.12 (C-3), 42.37 (C-4), 140.93 (C-5), 121.97 (C-6), 32.08 (C-7), 32.20 (C-8), 50.37 (C-9), 36.95 (C-10), 21.31 (C-11), 39.37 (C-12), 42.51 (C-13), 56.85 (C-14), 24.53 (C-15), 24.53 (C-16), 56.27 (C-17), 12.28 (C-18), 11.99 (C-19), 36.41 (C-20), 19.97 (C-21), 34.24 (C-22), 29.50 (C-23), 50.37 (C-24), 26.43 (C-25), 19.03 (C-26), 19.24 (C-27), 19.24 (C-28), 19.44 (C-29), 102.64 (C-1′), 75.36 (C-2′), 78.51 (C-3′), 71.72 (C-4′), 78.63 (C-5′) and 62.87 (C-6′).

In ^{1}H-NMR spectrum CH1 exhibited two tertiary methyl proton peaks at δ-0.68 (s, H-18), 0.85(s, H-19); three secondary methyl proton peaks at δ-0.91 (d, J = 6.42 Hz, H-26), 0.87(d, J = 6.42 Hz, H-27) and 1.01 (d, J = 6.5 Hz, H-21); one primary methyl proton peak at δ -0.90(t, J = 6.5 Hz, H-29); together with one anomeric proton peak at δ-5.07(1H, d, J = 8.22 Hz H-1′), one olefinic proton peak at δ-5.37(1H, bs, H-6) and one oxygen substituted methine proton peak at δ-3.89(1H, m, H-3). These proton data suggested that CH1 is a steroidal glycoside. In ^{13}C-NMR spectrum six carbon peaks at δ-102.64(C-1′), 75.36(C-2′), 78.51 (C-3′), 71.72 (C-4′), 78.625 (C-5′) and 62.87 (C-6′) are similar to those of methyl-β-D-glucose, which indicates the presence of a β-D-glucose unit (Zhao *et al*., 1992). CH1 is β-Sitosteryl-β-D-glucoside (Daucosterol) that is confirmed by comparing with the reported ^{13}C-NMR data of β-sitosterol (Gafur *et al*., 1997; Kojima *et al*., 1990) and methyl β-D-glucose. Previously β-sitosterol from *I. turpethum* was reported (Nasar, 1982), but not reported β-sitosterol-β-D-glucoside.

The petroleum ether, chloroform and ethyl acetate extracts and compound CH2 and EH4 showed moderate activities and CH1 showed little activities against various gram positive and gram negative bacteria (Table 1). Compounds CH2 and EH4 were showed MIC value at 64 μg mL^{-1} against *Bacillus subtilis*, *Shigella dysenteriae* and *Sarcina lutea* (Table 2). Compound CH2 and EH4 as well as extracts showed significant antifungal activity

Table 3: *In vitro* antifungal activities of extracts as well as of compounds CH1, CH2 and EH4

Test organisms	Diameter of zone of inhibition in mm						
fungi	I	II	III	CH1	CH2	EH4	Fcz-100
Aspergillus flavus	19	18	20	4	20	19	15
A. niger	15	17	19	0	25	23	25
A. Versicolor	31	28	21	5	28	21	24
Candida albicans	23	32	26	4	27	22	25

[Petroleum ether (I), chloroform (II) and ethyl acetate (III) extracts (each 200 μg/disc); CH1, CH2 and EH4 compounds (each 100 μg/disc) and Fcz-100 = Fuconazole (100 μg/disc)]

Table 4: Results of the brine shrimp lethality bioassay of compounds CH2, EH4 and standard ampicillin trihydrate (AT)

Concentration (C)	% of Mortality[a]		
(μg mL^{-1})	AT	CH2	EH4
5.0	46.7±1.7	16.2±1.3	16.4±1.9
10.0	53.3±1.2	27.7±1.5	30.3±1.2
20.0	66.7±1.9	36.3±1.9	40.2±1.3
40.0	76.7±1.3	47.3±1.7	47.1±2.3
80.0	92.1±1.4	54.2±2.1	58.4±1.7

[a]Each experiment was performed in triplicate and the results are expressed as the mean±standard deviation

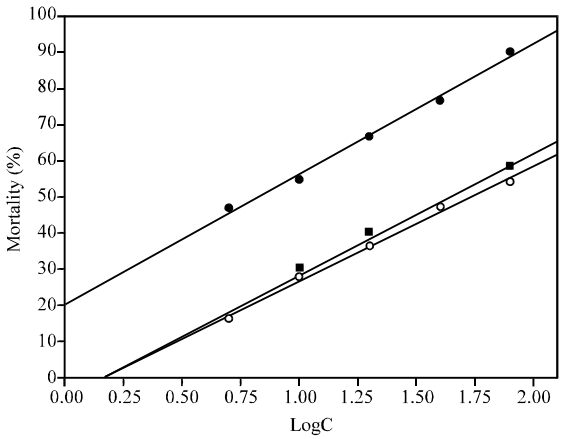

Fig. 2: Brine shrimp lethality bioassay of CH2, EH4 and AT (Ampicillin trihydrate). Graph plotted Log C versus % of mortality. ● AT, ■- EH4 and ⚬-CH2

(Table 3). The cytotoxic activity of CH2 and EH4 were showed 54.03 and 43.41 μg mL^{-1}, respectively while standard ampicillin trihydrate showed 6.83 μg mL^{-1} (Table 4 and Fig. 2).

From the antibacterial experiment, it is evident that the crude extract and compound CH2 and EH4 showed significant antibacterial activity but were less potent than standard kanamycin. In comparison to fuconazole; compounds CH2 and EH4 as well as extracts are strongly active against various fungi species. The significant antifungal activity of compounds CH2 and EH4 as well as extract indicate the presence of more potent antifungal metabolite in *I. turpethum*. This antibacterial and antifungal activity of compounds CH2 and EH4 and plant extracts are new report from *I. turpethum* and further justify the use of this plant in the management of microbial infection. In cytotoxicity experiment, it was shown that the compounds CH2 and EH4 are relatively less toxic than standard antibiotic. The cytotoxic action of a drug is exhibited by disturbing the fundamental mechanisms concerned with cell growth, mitotic activity, differentiation and function (Goodman *et al*., 1980). Although the exact mechanism of cytotoxic action of the compound could not be explained by these preliminary tests, it may be comment that the N-p-comaryl tyramine which is the new report from Convolvulaceae family may be used as a safe and effective chemotherapeutic agent. Where p-coumaric acid (Rashwan, 2002) and it's various derivatives showed goitrogenic activity in rats (Spasova *et al*., 2005; Khelfi *et al*., 2003). Thus, the findings of this investigation and previous investigations (Rashid *et al*., 2002) would give valuable support to make clinical trial as well as toxicity studies of the isolated antibacterial and antifungal metabolite (specially N-p-comaryl tyramine) to get a more potent antimicrobial agent in our present medical research. While the result of such a sub acute toxicological study will be reported elsewhere.

ACKNOWLEDGMENTS

We wish to thank National Institute of Health Science, Tokyo, Japan to provide the facilities of NMR and mass spectra analysis and to the authority of Beximco Pharma Limited, Bangladesh for providing the UV and IR spectra analysis. We also wish to thank to Professor A.T.M. Naderuzzaman, Department of Botany, University of Rajshahi and Bangladesh National Herbarium, Dhaka, Bangladesh to identify the plant.

REFERENCES

Alam, S., 2004. Synthesis, antibacterial and antifungal activities of some derivatives of 2-phenyl-chromen-4-one. J. Chem. Sci., 116: 325-331.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Budavari, S.M., J. O'Neil, A. Smith and P.E. Heckleman, 1989. The Merck index: An Encyclopaedia of Chemicals, Drugs and Biologicals. 11th Edn., Merck and Co. Inc., New Jersey, USA.

Gafur, M.A., T. Obata, F. Kiuchi and Y. Tsuda, 1997. Acacta concinna saponins. I structures of Prosapogenols, concinnosides A-F, Isolation from the alkaline hydrolysate of the highly polar saponin fraction. Chem. Pharm. Bull., 45: 620-625.

Goodman, L.S., A.G. Gilman and A. Gilman, 1980. Antiproliferative Agents and Immunosuppressive Drugs. The Pharmacological Basis of Therapeutics. Macmillan Pub. Co. USA 6th Edn., pp: 1299-1313.

Guan, Y., T.W. Miao Lin and J. Ye, 2006. Determination of pharmacological active ingredients in sweet potato (*Ipomoea batatas*) by capillary electrophoresis with electrochemical detection. J. Agric. Food Chem., 54: 24-28.

Khan, M.M.A.A., 1992. Physicochemical properties and mode of action of inhibitors of plant virus replication present in *Ipomoea turpethum* and *Scilla indica*. Z. Pfanzer Kranth pflazenschutz, 99: 71-73.

Khelfi, T.F., R.A. Taha, O.A. Badary, A. Lezzar and F.M.A. Hamada, 2003. Goitrogenic activity of p-coumaric acid in rats. J. Biochem. Mol. Toxicol., 17: 324-328.

Kirtikar, K.R. and D.B. Basu, 1994. Indian Medicinal Plant. Sing, M.P. (Ed.), Dehradun, India; 3:1730.

Kojima, H., N. Sato, A. Hantano and H. Ogura, 1990. Sterol glucosides from *Prunella vulgaris*. Phytochemistry, 29: 2351-2355.

Masako, A.B.E., Y. Ozawa, Y. Uda, F. Yamada, Y. Morimitsu, Y. Nakamura and T. Osawa, 2004. Antimicrobial activities of diterpene dialdehydes, constituents from Myoga (Zingiber mioga Roscoe) and their quantitative analysis. Biosci. Biotechnol. Biochem., 68: 1601-1604.

Mclaughlin, J.l. and J.E. Anderson, 1988. Brine shrimp and crown gal tumors: Simple bioassay for the discovery of plant antitumor agent, Bethesda, pp: 1-3.

Nasar, E.L.S.M.M., 1982. Coumarins of *Convolvulus lantus* and *C. arvensis*. Fitoterapia, 53: 189-191.

Rahman, A.T., M.K. Bhatti, F. Akter and M.I. Choudhury, 1992. Alkaloids of *Fumaria indica*. Phytochemistry, 31:2869-2872.

Rahman, A.A., A.T.M.Z. Azam and M.A. Gafur, 2000. Brine shrimp leathality bioassay with two flavonoids and extracts from *Clerodendrum indicum*. Pak. J. Pharmacol., 17: 1-6.

Rashid, M.H.O., M.A. Gafur, M.G. Sadik and A.A. Rahman, 2002. Antibacterial and cytotoxic activities of extracts and isolated compounds of *Ipomoea turpethum*. Pak. J. Biol. Sci., 5: 597-600.

Rashwan, O.A., 2002. New phenylpropanoid glucosides from *Eucalyptus amculata*. Molecules, 7: 75-80.

Reiner, R., 1982. Detection of Antibiotic Activity: Antibiotics, An Introduction. Roche Scientific Service, Switzerland, pp: 39-98.

Sahabuddin, 1999. Triterpenoids from the stem of *Operculina turpethum* L. Dhaka Univ. J. Biol. Sci., 8: 157-163.

Saieed, P., R.M. Reza, D. Abbas, R. Seyyedali and H. Allasghar, 2006. Inhibitory effects of *Ruta graveolens* L. extract on guinea pig liver aldehyde oxidase. Chem. Pharm. Bull., 54: 9-13.

Schimming, T., S.K. Jenett, K. Siems, L. Witte and E. Eich 2005. N1 N10-ditigloylspermine, a novel alkaloid from the seeds of *Ipomoea nil*. Pharmazie, 60: 958-959.

Spasova, M., K.V. Kortenska, I. Totseva, G. Ivanova, L. Georgiev and T. Milkova, 2005. Synthesis of Cinnamoyl and Hydroxylcinnamoyl Amino Acid Conjugates and Evaluation of Their Antioxidant Activity. J. Peptide Sci. (in press; www.interscience.wiley.com). DOI: 10.1002/psc.736.

Springer-Verlag, Berlin-Heidlberg, Newyork and Tokyo, 1983, Spectral data for structure determination, C120, C125, H135.

Srivastava, O.P., 1984. Technique for Evaluation of Antimicrobial Properties of Natural Products. In: The Use of Pharmacological Technique for Evaluation of Natural Products. Dhawan, B.N. and R.C. Srimal (Eds.)., UNESCO., New Delhi, pp: 72-79.

Zhao, G., Y. Hui, J.K. Rupprecht, J.L. Melaughlin and K.V. Wood, 1992. Additional bioactive compounds and trilobactin, a novel highly cytotoxic acetogenin, from the bark of *Asimina triloba*. J. Nat. Prod., 55: 347-356.