OPEM
www.opem.org

Oriental Pharmacy and Experimental Medicine 2008 **8(1)**, 97-102
DOI 10.3742/OPEM.2008.8.1.097

KHUP

Short Communication

Phytochemical and biological investigations of *Polygonum lanatum*

Sadia A Chowdhury[1,2], Mohammad H Sohrab[3], Mohammad R Haque[1], Choudhury M Hasan[1] and Mohammad A Rashid*[,1,2]

[1]Phytochemical Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh; [2]Centre for Biomedical Research, University of Dhaka, Dhaka-1000, Bangladesh; [3]Phytochemical Research Laboratory, Bangladesh Council for Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh

SUMMARY

A total of five compounds namely, 2-methylanthracene-9, 10-dione (1), 1-hydroxy-6-methylanthracene-9,10-dione (2), β-sitosterol (3), stigmasterol (4) and sitosterone (5) were isolated from the stem extracts of *Polygonum lanatum* for the first time. The structures of the isolated compounds (1-5) were established by extensive spectroscopic studies, including 2D NMR such as ^{1}H-^{1}H COSY, HSQC and HMBC studies. The crude extracts and purified compound (1) were screened for antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria and fungi by the disc diffusion method. The *n*-hexane and dichloromethane soluble partitionates of the methanolic extract revealed mild to moderate inhibition of growth of the test organisms. The cytotoxic potential of the extractives and compound 1 was also determined by using brine shrimp lethality bioassay, where the extractives demonstrated significant cytotoxic activities.

Key words: *Polygonum lanatum*; Polygonaceae; 2-methylanthracene-9; 10-dione; 1-hydroxy-6-methylanthracene-9; Antimicrobial activity; Cytotoxity

INTRODUCTION

Polygonum lanatum (Syn. *P. lanigerum*; Bengali name-Bishkatali; Family-Polygonaceae) is a herb, distributed in Eastern India, Bangladesh, Burma, Indonesia, Nepal and the Philippines. It is reputed for its anti-inflammatory, analgesic and diuretic activities (Saha *et al.*, 2005). Previous phytochemical studies with *Polygonum* species revealed the occurrences of a number of sterols (Fukuyama *et al.*, 1983), terpenoids (warburganal and related drimane-type sesquiterpenoids, Fukuyama *et al.*, 1982), flavonoid glycosides (Ahmed *et al.*, 1988; Khoda *et al.*, 1990), coumaryl glycoside (hydropiperoside, Fukuyama *et al.*, 1983), quercetin glycosides (Fukuyama *et al.*, 1983), lignans (Kim *et al.*, 1994) and quinones (Fukuyama *et al.*, 1983; 6-methoxyplumbagin, Al-Hazimi and Haque, 2002). We, herein, report the isolation of 2-methylanthracene-9, 10-dione (1) (Chakraborty *et al.*, 1978), 1-hydroxy-6-methylanthracene-9, 10-dione (2) (Bhargava *et al.*, 1991), β-sitosterol (3) (Morales *et al.*, 2003), stigmasterol (4) (IKhan, 1991) and sitosterone (5)

*Correspondence: Mohammad A Rashid, Phytochemical Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh. Tel: +88028612069, 966190073, Ext. 4363, 8137; Fax: +88028612069; E-mail: rashidma @aitlbd.net.

(Joshi *et al.*, 1974) from *P. lanatum* for the first time. The results of antimicrobial screening of the crude methanolic extract and its *n*-hexane and dichloromethane soluble fractions as well as the purified compound 1 against a wide range of Gram-positive bacteria, Gram-negative bacteria and fungi by disc diffusion method (Bauer *et al.*, 1966) and cytotoxicity activity by brine shrimp lethality bioassay (McLaughlin, 1982; Persoone, 1988).

MATERIALS AND METHODS

General

^{1}H and ^{13}C NMR spectra were acquired using the Ultra Shield Bruker DPX 400 NMR instrument, and the chemical shifts are reported in ppm with respect to TMS or residual non deutarated solvent signals.

Plant material

The stems of *P. lanatum* were collected from Narshindi district in the month of July 2005. The plant was identified by Dr. Mahbuba Khanum, Principal Scientific Officer, Bangladesh National Herbarium, Dhaka**,** where a voucher specimen has been deposited (DACB Accession no. 31, 286). The stems were first sun dried and then ground into a coarse powder using a grinding machine.

Extraction and isolation

The air-dried and powdered plant material (503 mg) was separately extracted to exhaustion in a Soxhlet apparatus with *n*-hexane, then dichloromethane (CH_2Cl_2) and finally with methanol (MeOH). The individual extractive was filtered through fresh cotton bed and finally with Whatman no.1 filter paper. The filtrates were concentrated with a rotary evaporator at low temperature (40 - 50^{o}C) and reduced pressure to provide *n*-hexane (4.25 mg), dichloromethane (2.0 mg) and methanol (4.0 mg) soluble materials.

The *n*-hexane soluble materials were subjected to preparative thin layer chromatography (PTLC) over silica gel (mobile phase: toluene-ethyl acetate; 80:20 with few drops of acetic acid; multiple developments; thickness of plates: 0.5 mm). Repeated purification of the extractive yielded compound 3 (1.5 mg). Similar purification of the dichloromethane extract of *P. lanatum* provided compound 5 (1.6 mg).

The methanol soluble extract was fractionated by column chromatography over silica gel (Kieselgel 60, mesh 70 - 230). The column was eluted with *n*-hexane, dichloromethane and methanol mixtures of increasing polarities to provide 11 fractions. Compound 1 (10.5 mg) was isolated from fraction-2 by PTLC of over silica gel F_{254} (mobile phase: 12.5% ethyl acetate in toluene). Compound 4 (6.2 mg) was obtained from fraction-4 by preparative TLC over F_{254} silica gel using 25% ethyl acetate in petroleum ether as the mobile phase. Fractions-5, 6 and 7 were mixed together and subjected to further column chromatography over silica gel (Kieselgel 60, mesh 70 - 230). The column was eluted with *n*-hexane, ethyl acetate and methanol mixtures of increasing polarities to provide 9 fractions designated by (A - I). Preparative TLC of fraction-A over silica gel using 12.5% ethyl acetate in toluene provided compound 2 (3 mg). Compounds 1 and 3 were isolated through UV detection at short (254 nm) and long (366 nm) wavelengths, while the remaining compounds on TLC plate were visualized by spraying vanillin/H_2SO_4 followed by heating.

2-Methylanthracene-9,10-dione (1): Yellowish mass; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 (2H, m, H-5, H-8), 8.20 (1H, d, *J* = 8.0 Hz, H-4), 8.09 (1H, br. s, H-1), 7.78 (2H, m, H-6, H-7), 7.58 (1H, br. d, *J* = 8.0 Hz, H-3), 2.53 (3H, s, CH_3-2); ^{13}C NMR (100 MHz, $CDCl_3$): 183.5 (C-9), 183.0 (C-10), 145.3 (C-2), 135.0 (C-3), 134.0 (C-6, C-7), 133.6 (C-8a, C-9a), 133.5 (C-10a), 131.3 (C-4a), 127.5 (C-1, C-4), 127.2 (C-5, C-8), 21.9 (Me-2); HMBC: H-8/C-9; H-5/C-10; H-4/C-2, C-10, C-9a; H-3/C-1, C-4a; H-1/C-3, C-4a, C-9; CH_3/C-1, C-2, C-3.

1-Hydroxy-6-methylanthracene-9,10-dione (2): Yellow gum; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.67

(1H, s, OH-1), 8.30 (1H, m, H-4), 8.21 (1H, d, *J* = 8.2 Hz, H-8), 8.09 (1H, br. s, H-5), 7.82 (1H, t, *J* = 8.0 Hz, H-3), 7.65 (1H, br. d, *J* = 8.0 Hz, H-2), 7.60 (1H, br. d, *J* = 8.2 Hz, H-7), 2.54 (3H, s, CH_3-6).

β-sitosterol (3): White crystals; ^{1}H NMR data identical to previously reported values (Morales *et al*., 2003).

Stigmasterol (4): Colorless needles; ^{1}H NMR data was in close agreement to published data (IKhan, 1991).

Sitosterone (5): White gum; ^{1}H NMR data was identical to published value (Joshi *et al*., 1974).

Biological screenings

Antimicrobial assay

The disc diffusion method (Bauer *et al*., 1966) was used to test antimicrobial activity against thirteen bacteria and three fungi (Table 1). Solutions of known concentration (mg/ml) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amounts of the test substances using micropipette. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs and blank discs (impregnated with solvents) were used as positive and negative control. These plates were then kept at low temperature (4°C) for 24 h to allow maximum diffusion. There is a gradual change of test materials concentration in the media surrounding the discs. The plates were then incubated at 37°C for 24 h to allow maximum growth of the organisms. The test material having antimicrobial activity, inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antimicrobial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiment is carried out three times and the mean of the readings is required (Bayer *et al*., 1966). Standard disc of kanamycin (30 μg/disc) was used for comparison purpose.

Table 1. Antimicrobial activity of extractives of *P. lanatum* and Kanamycin

Test bacteria and fungi	HEX	DCM	ME	Compound 1	Kanamycin
Bacteria					
Bacillus cereus	13	13	-	ND	39
B. megaterium	12	11	-	ND	32
B. subtilis	-	-	-	ND	20
Staphylococcus aureus	7	7	-	ND	22
Sarcina lutea	10	8	-	13	20
Escherichia coli	7	8	ND	7	23
Pseudomonas aeruginosa	11	10	8	ND	26
Salmonella paratyphi	12	13	10	9	30
S. typhi	8	10	ND	ND	20
Shigella boydii	12	11	9	ND	26
S. dysenteriae	8	7	ND	8	24
Vibrio mimicus	7	8	ND	ND	24
V. parahemolyticus	15	11	-	ND	38
Fungi					
Candida albicans	9	10	ND	8	24
Aspergillus niger	14	12	7	ND	32
Sacharomyces cerevacae	10	10	-	ND	30

"-" Indicates 'no activity' and "ND" indicates 'not done'.

Cytotoxicity study

Brine shrimp lethality bioassay (McLaughlin, 1982; Persoone, 1988) technique was applied for the determination of cytotoxic property of various fractions of the crude extract and compound 1.

Preparation of positive control group

Vincristine sulphate was used as the positive control. Measured amount of the vincristine sulphate was dissolved in DMSO to get an initial concentration of 20 μg/ml from which serial dilutions were made using DMSO to get 10 μg/ml, 5 μg/ml, 2.5 μg/ml, 1.25 μg/ml, 0.625 μg/ml, 0.3125 μg/ml, 0.15625 μg/ml, 0.078125 μg/ml, 0.0390 μg/ml. Then the positive control solutions were added to the premarked vials containing ten living brine shrimp nauplii in 5 ml simulated sea water to get the positive control groups.

Preparation of negative control group

100 μl of DMSO was added to each of three pre-marked glass vials containing 5 ml of simulated sea water and 10 shrimp nauplii to use as control groups. If the brine shrimps in these vials show a rapid mortality rate, then the test is considered as invalid as the nauplii died due to some reason other than the cytotoxicity of the compounds.

Counting of nauplii

After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration.

RESULTS AND DISCUSSION

Repeated chromatographic separation and purification of the *n*-hexane, dichloromethane and methanolic extracts of the stem of *P. lanatum* provided a total of five compounds (1 - 5), the structures of which were determined by extensive NMR spectral analysis.

The ^{13}C NMR spectrum of compound 1 displayed 15 carbon resonances, including two carbonyl group signals at δ 183.0 and 183.5. The HSQC and DEPT experiments indicated that 8 out of the 15 carbons had attached protons. The DEPT 135 spectrum revealed the presence of seven aromatic methene carbons and a methyl group resonance. The ^{1}H and ^{13}C NMR spectral data of this compound demonstrated the general features of substituted anthraquinone skeleton (Chakraborty *et al.*, 1978). The ^{1}H NMR spectrum showed two multiplets, each integrating for two protons, at δ 7.78 and δ 8.30 indicative of a typical 1, 2-disubstituted aromatic ring. Two doublets (J = 8.0 Hz), each of one proton integrating, centered at δ 7.58 and δ 8.20 could be assigned to the two *ortho*-coupled aromatic protons. A one proton singlet at δ 8.09 was attributed to H-1. A singlet integrating for three protons at δ 2.53 suggested the presence of an aromatic methyl group, whose presence was confirmed from the ^{13}C NMR signal at δ 21.9. Analysis of one- and two-dimensional NMR spectra including COSY, HSQC and HMBC data disclosed the structural features of 1 (Fig. 1). The position of the aromatic methyl

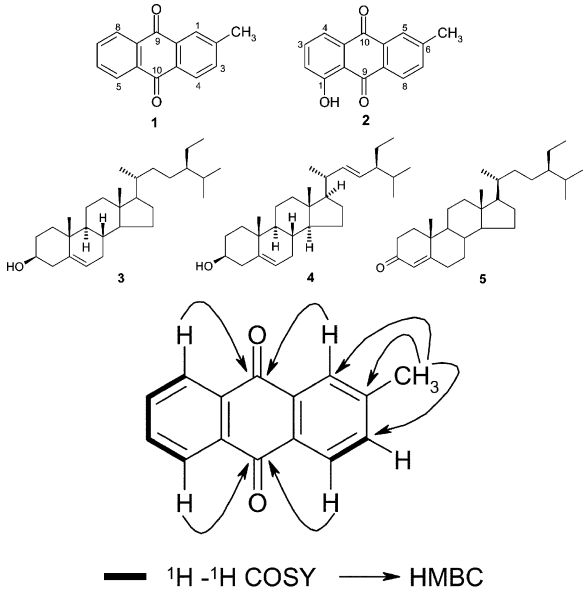

Fig. 1. Key ^{1}H-^{1}H COSY and HMBC correlations observed in 1.

group at C-2 was unambiguously determined by HMBC correlations from the methyl proton to C-1, C-2 and C-3. On this basis, compound 1 was identified as 2-methylanthracene-9,10-dione, previously reported from *Clausena heptaphylla* (Chakraborty *et al*., 1978).

The ^{1}H NMR spectral data of compound 2 was almost identical to that of 2-methylanthracene-9,10-dione (1) and 4-deoxyanthrakunthone (Khoda *et al*., 1990), suggesting a close structural similarity among these compounds. Thus, it revealed the presence of six protons in the aromatic region, a methyl group and a phenolic hydroxyl functionality. The presence of a one-proton broad signals at δ 8.30 could be ascribed to the aromatic proton H-5, while the triplet at δ 7.81 (J = 8.0 Hz) and a broad doublet at δ 7.65 were assigned to H-3 and H-2, respectively. The doublets (J = 8.2 Hz), each integrating for one proton, centered at δ 7.60 and δ 8.21 could be assigned to *ortho*-coupled aromatic protons at C-7 and C-8, respectively. A singlet integrating for three protons at δ 2.54 indicated the presence of an aromatic methyl group at C-2. The sharp singlet at δ 12.67 could be ascribed to the chelated phenolic hydroxyl proton at C-1. On the basis of the above spectral features compound 2 characterized as 1-hydroxy-6-methylanthracene-9,10-dione (2), previously known to occur in *Tectona grandis*. The identity of compound 2 was further confirmed by comparison of its spectral data with published values (Bhargava *et al*., 1991).

Compounds 3, 4 and 5 were identified as *β*-sitosterol, stigmasterol and sitosterone, respectively by comparison of their ^{1}H NMR spectral data with reported values as well as by co-TLC with authentic samples.

The *n*-hexane (HEX) and dichloromethane (DCM) soluble fractions (500 μg/disc) and pure compound 1 (300 μg/disc) were screened against 13 test bacteria and 3 fungi and exhibited mild to moderate antimicrobial activity against most of the test organisms whereas, the methanolic (500 μg/disc) crude extract showed poor antimicrobial activity in most cases (Table 1). The zone of inhibition produced by *n*-hexane, dichloromethane and methanol extracts were found to be 07 - 15 mm, 07 - 13 mm and 07 - 10 mm respectively. The *n*-hexane soluble fraction showed mild activity against *Bacillus megaterium*, *Pseudomonas aeruginosa*, *Salmonella paratyphi* & *Shigella boydii* and moderate activity against *Bacillus cereus*, *Vibrio parahemolyticus* and *Aspergillus niger*. The dichloromethane soluble fraction showed mild activity against *Bacillus megaterium*, *Shigella boydii*, *Vibrio parahemolyticus* & *Aspergillus niger* and moderate activity against *Bacillus cereus* & *Salmonella paratyphi*. *B. subtilis* was found to be resistant to the test samples.

In the cytotoxicity study, the *n*-hexane and dichloromethane soluble fractions were found to be highly lethal to brine shrimp nauplii. As a result, LC_{50} values could not be determined. The LC_{50} of methanol extract and the purified compound 1 were found to be 3.35 μg/ml and 114.88 μg/ml respectively. Although all the test samples were lethal to brine shrimp nauplii, the hexane and dichloromethane extracts were comparatively more active than the methanol extract and compound 1. This suggests that the hexane and dichloromethane extract may contain additional cytotoxic agents.

Although the extractives showed strong cytotoxicity against brine shrimp nauplii, none of them demonstrated significant inhibition of growth of the test microorganisms. This was probably due to the development of partial or complete resistance of the microorganisms against the test samples, which might be the result of the indiscriminate use of antibacterial agents.

ACKNOWLEDGEMENTS

We wish to thank the Bangladesh Council for Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh for NMR studies and the Ministry of Science, Information & Communication Technology for partial financial support to two of us (C.M.H.

and M.A.R.) to carry out the research. S.A.C. is grateful to Mr. Kh. M. Rahman for his assistance in the laboratory works.

REFERENCES

Ahmed M, Khaleduzzaman M, Rashid MA. (1988) Chalcone derivatives from *Polygonum lapathifolium*. *Phytochemistry* **27**, 2359-2360.

Al-Hazimi HMA, Haque SN. (2002) A new napthoquinone from *Polygonum aviculare*. *Nat. Prod. Lett.* **16**, 115-118.

Bauer AW, Kirby WMM, Sherries JC, Tuck M. (1966) Antibiotic susceptibility testing by a standardized disc diffusion method. *Am. J. clin. Pathol*. **45**, 493-496.

Bhargava S, Jain S, Suri A, Singh P. (1991) Further studies on anthraquinone derivatives from *Tectona grandis*. *J. Indian Chem. Soc.* **68**, 631-632.

Chakraborty DP, Islam A, Roy S. (1978) 2-methyl anthraquinone from *Clausena heptaphylla*. *Phytochemistry* **17**, 2043.

Fukuyama Y, Sato T, Miura I, Asakawa Y, Takemoto T. (1982) A potent cytotoxic warburganal and related drimane-type sesquiterpinoids from *Polygonum hydropiper*. *Phytochemistry* **21**, 2895-2898.

Fukuyama Y, Sato T, Miura I, Asakawa Y, Takemoto T. (1983) Hydropiperose, a novel coumaryl glycoside from the root of *Polygonum hydropiper*. *Phytochemistry* **22**, 549-552.

Ikhan, R. (1991) Natural product: A laboratory guide, 2nd Edition, Academic Press, N.Y., USA.

Joshi KC, Bansal RK, Shingh P. (1974) Mass and NMR spectral studies of sitost-4-ene-3-one from *Tabebuea rosea*. *Indian J. Chem.* **12**, 903-904.

Kohda H, Niwa A, Nakamoto Y, Takeda O. (1990) Flavanoid glycisides from *Polygonum tinctorium*. *Chem. Pharm. Bull.* **38**, 523-524.

Kim HJ, Woo ER, Park H. (1994) A novel lignan and flavonoids from *Polygonum aviculare*. *J. Nat. Prod.* **57**, 581-586.

McLaughlin JL. (1982) Brine shrimp: a convenient general bioassay for active constituents. *Planta Med.* **45**, 31-32.

Morales G, Sierra P, Mancilla A, Paredes A, Loyola LA, Gallardo O, Borquez J. (2003) Secondary metabolites from four medicinal plants from northern Chile: antimicrobial activity and biotoxicity against *Artemia salina*. *J. Chil. Chem. Soc.* **48**, 2.

Persoone G. (1988) Proceedings of the international symposium on brine shrimp, *Artemia salina*. University Press, Wittern, Belgium, p. 1-3.

Saha A, Chowdhury KK, Bachar SC, Roy SC, Kundu JK. (2005) Anti-inflammatory, analgesic and diuretic activity of *Polygonum lanatum*. *Pak. J. Pharm. Sci*. **18**, 13-18.