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Antibacterial Activity of 8-(4'-methoxybenzyl)-xylopinine from *Stephania glabra* Tubers

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Abstract: *Stephania glabra* (Roxb.) Miers (Menispermaceae) is a large, climbing shrub, indigenous to lower Himalaya of India. This plant has long been used for the treatment of asthma, tuberculosis, dysentery, hyperglycemia, cancer, fever, intestinal complaints, sleep disturbances and inflammation in India. The study aim is to isolate the chemical constituents from the plant tubers and to evaluate antimicrobial activity of isolated constituents. Ethanolic extract from tubers was subjected to column chromatography using silica gel as adsorbent for isolation of chemical constituents. The agar diffusion method was adopted for antimicrobial activity to determine IZD and MICs. A tetrahydroberberine alkaloid named 8-(4'-methoxybenzyl)-xylopinine has been isolated from the column eluent of $CHCl_3$:MeOH (1:9). The compound showed antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Eschericha coli* and *Salmonela typhi* with IZD range of 8-19 mm at MIC of 25-100 μg mL^{-1}. Erythromycin (10 μg mL^{-1}) was used as standard antibiotic. Present study concludes that 8-(4'-methoxybenzyl)-xylopinine have significant antibacterial activity.

Key words: *Stephania glabra*, Menispermaceae, tetrahydroberberine, antibacterial activity, alkaloid

INTRODUCTION

Stephania glabra (Roxb.) Miers (Menispermaceae) is a large, climbing shrub, indigenous to lower Himalaya of India. The plant has long been used for the treatment of asthma, tuberculosis, dysentery, hyperglycemia, cancer, fever, intestinal complaints, sleep disturbances and inflammation in India (Semwal *et al*., 2010a). Recently, we have reported three novel alkaloids from the tubers of this plant (Semwal and Rawat, 2009a, b; Semwal *et al*., 2010b). The extract obtained from the plant tubers and some isolated compounds were also screened for various biological activities including antimicrobial, antidiabetic, antipyretic and analgesics (Semwal *et al*., 2009; Semwal *et al*., 2010c; Semwal *et al*., 2011). This study describes the isolation and structure elucidation of a tetrahydroberberine alkaloid 8-(4'-methoxybenzyl)-xylopinine (1) along with its antibacterial activities for the first time from this source.

MATERIAL AND METHODS

General: Mp: uncorr. UV spectrum on Perkin-Elmer, Lambda- 25 spectrometer in MeOH. IR on Perkin-Elmer, Spectrum RX I FT-IR spectrometer (KBr discs). NMR spectra were obtained on JEOL NMR spectrometer (400 MHZ for ^1H and 100 MHZ for ^{13}C NMR) in DMSO-d_6 with TMS as int. standard. Mass spectrum was recorded on Finnigan MAT spectrometer (CA, USA, xcalibur ver-2 software).

Plant material: Fresh tubers (10 kg) were collected from Village Chaka, District Tehri Garhwal, India during October 2006 and identified by Prof. R.D. Gaur, Department of Botany H.N.B. Garhwal University Srinagar. A voucher specimen (GUH-17600) of the plant was deposited in the Departmental herbarium for future records.

Extraction and isolation: Air dried finely powdered tubers were extracted exhaustively with 95% ethanol at 30-50°C (for 15 h, 3 times) on a heating mantle. The extraction mixture was filtered and solvent evaporated to dryness under reduced pressure to yield black brown residue (200 g). It was chromatographed by pre-adsorbed onto silica gel (200 g) and then added to the top of the column prepared by using 500 g silica gel (Merck, 60-120 mesh) in $CHCl_3$. Elution was first started with $CHCl_3$ and then with $CHCl_3$: MeOH = 49:1, 24:1, 47:3, 23:2, 9:1, 22:3, 43:7, 21:4 and 41:9. The fractions obtained from column were collected every 100 mL and combined on the basis of TLC analysis. The elution with $CHCl_3$: MeOH (9:1) afforded compound 1.

8-(4'-methoxybenzyl)-xylopinine (1): White powder (MeOH, 96 mg); mp: 170-172°C; $[\alpha]_D^{20}$: -115° (c 0.5, $CHCl_3$); MF. $C_{29}H_{33}NO_5$; UV λ_{max}^{MeOH} : 315, 286, 252, 210 nm; IR: υ_{max}^{KBr} 2942, 1611, 1231, 937 cm^{-1}; ^1H and ^{13}C NMR data: (Table 1), LCMS: (m/z, rel. abun.): 476 (6) $[M+H]^+$, 475 (20) $[M]^+$, 241 (54), 210 (37), 192 (100), 149 (18), 117 (26); Elemental analysis:

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(found C, 73.82 H, 6.64; N, 2.83; O, 16.71%; calculated for $C_{29}H_{33}NO_5$: C, 73.24; H, 6.99; N, 2.95; O, 16.82%).

Antibacterial evaluation: Antimicrobial study of compound 1 was carried out by the agar disc diffusion method (Cheebroug, 2000) against four test microorganisms *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Eschericha coli* and *Salmonela typhi*. Test organisms has isolated from different culture media were studied for Inhibition Zone Diameter (IZD) and Minimum Inhibitory Concentration (MIC) (Table 2). Nutrient broth tubes (for bacterial species incubated at 37°C for 24 h) were prepared for MIC determination. MIC was determined as the least concentration of compound 1 inhibiting the growth of the test organisms (Tarfa *et al*., 2004). Erythromycin (Alembic Limited, Vadodara) has been used as standard antibiotic.

Table 1: ^{13}C (100 MHZ) and ^{1}H NMR (400 MHZ) data of 1 in DMSO d^6

Position	δ_C ppm	δ_H ppm (J Hz)	Position	δ_C ppm	δ_H ppm (J Hz)
1	112.7	6.93 s	13	26.6	2.82 d (2.5)
1a	126.0	-	13a	72.3	3.21 d (3.8)
2	153.1	-	1'	126.1	-
3	153.8	-	2'	106.5	6.68 d (4.1)
4	109.3	7.18 s	3'	109.3	6.71 d (1.3)
4a	127.5	-	4'	158.0	-
5	27.2	2.74 t (3.2, 2.2)	5'	108.9	6.74 d (4.1)
6	63.2	3.92 t (1.2, 1.2)	6'	106.9	6.80 d (6.4)
8	74.3	3.82 m	7'	21.2	2.48 s
8a	119.8	-			
9	112.0	7.56 s	OMe-2	55.4	3.90 s
10	148.1	-	OMe-3	55.6	4.03 s
11	152.7	-	OMe-10	56.9	4.07 s
12	115.3	6.85 s	OMe-11	56.3	3.88 s
12a	122.4	-	OMe-4'	55.9	4.12 s

Table 2: Antibacterial activity of compound 1

	MIC (μg mL^{-1})		
	----------		IZD (mm)±SD
Test organisms	1		Erythromycin (10 μg mL^{-1})
Pseudomonas aeruginosa	50	12±0.54	17±0.33
Escherichia coli	100	08±0.35	18±0.33
Salmonela typhi	25	19±0.35	13±0.33
Staphylococcus aureus	25	18±0.26	15±0.33

RESULTS AND DISCUSSION

Compound 1 was isolated as white amorphous powder, mp. 170-172°C, proposed molecular formula $C_{29}H_{33}NO_5$ from molecular ion peak at m/z 475 in its LCMS. It gave positive tests with Wagner's, Dragendroff's and Hager's reagents indicated its alkaloidal nature. Figure 1 presented chemical structure of compound 1.^{1}H NMR express the evidence for five methoxy groups (δ 3.88, 3.90, 4.03, 4.07 and 4.12, all singlet) which was further supported by ^{13}C NMR spectrum (δ 55.4, 55.6, 55.9, 56.3 and 56.9), eight close downfield signals between δ 6-8 were due to aromatic protons. ^{13}C NMR and DEPT spectra showed 29 signals for ten quaternary (>C<), ten tertiary (>CH-), four secondary (>CH_2) and five primary (-CH_3) carbon atoms. All these spectroscopic findings were corroborated to a tetrahydroberberine alkaloid which was found similar in all respects to that of 8-(4'-hydroxybenzyl) xylopinine (Xu and Liu, 1999) except one additional O-methyl group in 1. The position of additional O-methyl group was confirmed by the HMBC correlation of δ 4.12 (OCH_3-4') to δ 158.0 (C-4') and by the mass fragmentation pattern (Fig. 2) which showed an abundant peak at m/z 149 (18%) was due to $C_9H_{10}NO^+$ ion, after the loss of one methoxyl radical it gave $C_8H_8N^+$ ion (m/z 117, 26%) followed by

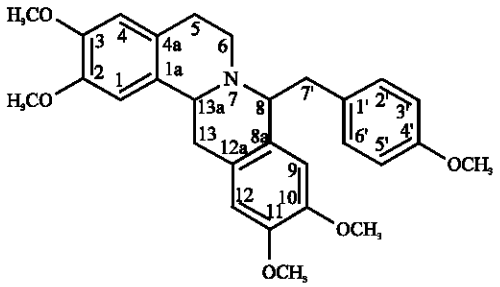

Fig. 1: Chemical structure of compound 1

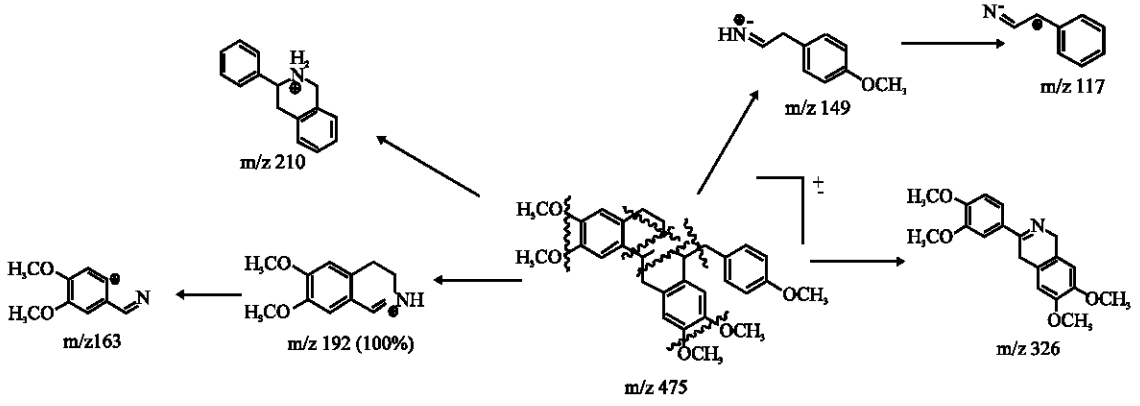

Fig. 2: Proposed mass fragmentation of compound 1

hydrogen transfer. These findings revealed that methoxy group must located at C-4', similar to the position of hydroxyl group in 8-(4'-hydroxybenzyl) xylopinine. Hence, the compound 1 was characterized as 8-(4'-methoxybenzyl) xylopinine.

Compound 1 showed potent antibacterial activity against *S. aureus* and *S. typhi* with IZD of 18 and 19 mm, respectively at MIC of 25 μg mL^{-1} whereas, it showed a mild activity with IZD of 8 mm at MIC of 100 μg mL^{-1} against *E. coli* (Table 2). The results were compared with standard antibiotic erythromycin (10 μg mL^{-1}).

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