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Isolation of Secondary Metabolites from the Roots of *Salix Babylonica*

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Abstract : Chemical examination of ethanolic extract of *Salix babylonica*, root, led to isolation of 2-(Hydroxymethyl) phenyl β -D-glucopyranoside (1) and 2-[(benzoyloxy)methyl]-4-hydroxyphenyl β -D-glucopyranoside 3-Benzoate (2) with previously known compounds β -Sitosterol, kaempferol 7-O-glucoside and apigenin 7-O-galactoside. Compounds 1 and 2 had been isolated for the first time from this plant. The structures of the compounds had been elucidated with the help of spectral and chemical studies.

Key words – Glycoside, metabolites, *Salicaceae*, *Salix babylonica*.

1. INTRODUCTION

The green plants are the storehouses of many chemical components which are called metabolites. The metabolites have a capability to convert simpler inorganic compounds into complex organic compounds. The complex organic compounds are used for several metabolic activities [1]. *Salix babylonica* (weeping willow), belongs to family *Salicaceae*. *Salix babylonica* is a deciduous, short-lived tree to 20 m tall. Stem is furrowed, usually dividing near the ground, open crown, pendulous branches. Grey-black bark. Leaves lanceolate to linear lanceolate, 9-16 cm long, 0.5-1.5 cm wide, finely serrated margins, slightly shiny, dark green, grey-green with distinctly reticulate venation beneath. Leaf stalk 5-10 mm long, pubescent. Flowers in short, terminal catkins on leafy peduncles. Catkin is 1.8-3 cm long and 0.5-1 cm wide in male. Catkin is 1.5-2.5 cm long and 0.5 cm wide in female. Fruit is a brown yellowish capsule. Bark and leaves have astringent and tonic properties. Young twigs and catkins are antipyretic. A leaf infusion is given to rheumatics [2]. Earlier studies showed that different compounds had been isolated from the genus such as terpenoids [3], catechins [4], lignans [5], flavones [6,7], phenolic compounds [8], benzyl ester of gentisic acid 2'-O-acetyl β -D-glucoside, trichocarpin, salicin, kaempferol 7-O-glucoside, apigenin 7-O-galactoside, luteolin 4'-O-glucoside and a terephthalic acid ester [9], tritetracosane, octadecenoic acid-1,2,3-propanetriyl ester, hexadecanoic acid-methyl ester, and 1,3-dioxane-4-(hexadecyloxy)-2-pentadecyl [10], 2',5-dihydroxy-3-methoxyflavone-7-O- β -D-glucopyranoside, β -sitosterol, 2,6-dihydroxy-4-methoxy acetophenone, eugenol-1-O- β -D-glucopyranoside, 1-O- β -D-(3'-benzoyl) salicyl alcohol and luteolin-7-O- β -D-glucopyranosyl-(1-6)-glucopyranoside [11]. *Salix babylonica* plant extract was also tested for its insecticidal [12], fungicidal [13] and



antioxidant [14] activities. The present paper illustrates the isolation and structure elucidation of the compound 1 and compound 2 from the alcoholic extract of *Salix babylonica* roots together with four known compounds.

2. EXPERIMENTAL

2.1. Materials and methods

Melting points had been recorded with the help of Perfit melting point apparatus. Perkin-Elmer Lambda-25 spectrophotometer was used to measure the UV spectra in methanol. A Perkin-Elmer Spectrum RX1 FT-IR spectrometer was used for detection of IR spectra over KBr discs. NMR spectra were recorded by Bruker Avance 300 and 500 spectrometers (300 MHz for ^1H and 125 MHz for ^{13}C using CDCl_3 as solvent and TMS as internal standard). Atmospheric Pressure Chemical Ionization Mass Spectrometer (APCIMS) technique was used for detection of MS. Column chromatography technique was to be performing over silica gel. Thin Layer Chromatography (TLC) had been carried out on silica gel (10-40 μ) pre coated plates, these plates had been sprayed by 7% H_2SO_4 solution for visualization of spots.

2.2. Plant material

Salix babylonica roots were collected from Pauri Garhwal, Uttarakhand, India and identified from the Plant Identification Laboratory, Department of Botany, Hemwati Nandan Bahuguna Garhwal University Srinagar Garhwal, Uttarakhand, India.

2.3. Extraction and isolation

The roots of the plant were air dried at 25°C temperature under shade and later grounded into fine powder. Alcoholic extract was prepared by putting the plant material (2 kg) in Soxhlet apparatus with 95% Ethanol. The ethanolic extract was filtered and then evaporated until it becomes thick paste. The extract was then fractionated through column chromatography using chloroform: methanol as eluting solvent. The polarity of solvent was gradually increased by addition of methanol. The repeated column chromatography afforded Compound 1 and 2 together with previously isolated compounds β -Sitosterol, Querceitin, kaempferol 7-O-glucoside and apigenin 7-O-galactoside.

2.4. Compound 1

It was crystallized from chloroform as white amorphous powder. M.P. $203-205^\circ\text{C}$, Molecular formula $\text{C}_{13}\text{H}_{18}\text{O}_7$, Molecular weight 286 amu, IR ($\lambda_{\text{max}}^{\text{KBr}}$) cm^{-1} , 3240, 3040, 1895, 1660, 1450, 1250, 1190, 915, 850, APCIMS 325 $[\text{M}+\text{K}]^+$, 309 $[\text{M}+\text{Na}]^+$, 286 $[\text{M}]^+$, 285 $[\text{M}-\text{H}]^+$, 265, 214, 158, 123 $[\text{M}-\text{H}-162]^+$, 113, 102. The ^1H -NMR and ^{13}C -NMR spectral data have been given in table 1.

2.5. Compound 2

It was crystallized from methanol as amorphous powder, M.P. $317-319^\circ\text{C}$, Molecular formula $\text{C}_{27}\text{H}_{36}\text{O}_{10}$, Molecular weight 510 amu, UV ($\lambda_{\text{max}}^{\text{MeOH}}$) 282 nm (characteristics to phenolic group), IR ($\lambda_{\text{max}}^{\text{KBr}}$) cm^{-1} 3420, 1710, 1600, 1500, 1277 and 1211. The ^1H -NMR and ^{13}C -NMR spectral data have been given in table 2

3. RESULTS AND DISCUSSION

3.1. Compound 1

It has been crystallized from chloroform as white amorphous powder, M.P. 203-205⁰C. Its molecular formula had been assigned as C₂₇H₃₆O₁₀. The molecular ion peak observed at m/z 285[M-H]⁺, other peaks observed at 265, 214, 158. The peak at 123[M-H-162] showed the loss of one hexosyl unit from molecular ion peak, thus the mass loss of 162 amu showed the presence of hexose sugar in molecule. It also showed other fragmentation peaks at m/z 325[M+K]⁺, 309[M+Na]⁺ in its APCIMS. The IR ($\lambda_{\max}^{\text{KBr}}$) cm⁻¹ showed characteristic absorption band at 3240 cm⁻¹ which indicate the presence of hydroxyl group in the compound. The ¹H-NMR spectrum of the compound showed two AB type doublets (J=8.8 Hz) each for 2H in aromatic region at δ 8.00 and 7.36, which revealed the disubstituted aromatic nature of the compound. A sharp singlet at δ 9.00 was assigned for OH group present in the compound. A doublet integrating for one proton at δ 5.72 (J=7.0 Hz) was attributed to the anomeric proton of sugar moiety present in the molecule.

¹³C-NMR spectrum of compound showed thirteen carbon resonance lines (of which two were of double intensity). It also shows three tertiary and four secondary carbon atoms. The downfield chemical shift of C-4 at δ 163 as compared to other aromatic carbon atom showed the position of sugar is attached at C-4' carbon atom of the compound other six aromatic signals had observed at δ 131.6 (C-2,6), 117 (C-3,5), 132.6 (C-1) and 163 (C-4) thus on the basis of above discussion it was identified as **2-(Hydroxymethyl) phenyl β -D-glucopyranoside**. [Fig.1]

3.2. Compound 2

The APCIMS spectrum showed a molecular ion peak observed at m/z 510[M+H]⁺ which resulting to the molecular formula C₂₇H₂₆O₁₀. It exhibit UV absorption band at 282 nm was due to the presence of phenolic group. There was an intense IR absorption band at 1710 cm⁻¹ which indicate the presence of an ester functional group, other strong absorption band observed at 3420 (OH), 1600-1500 (C=C, aromatic), 1277 and 1211 (C-O-C) cm⁻¹ showed the presence of hydroxyl group, aromatic double bond and ether linkage in the molecule, while the broad (C-O) stretching bands in the region of 1121-1030 cm⁻¹ suggested its glycosidic nature. The molecular ion peak observed at m/z 510[M]⁺ by APCIMS spectra of the compound, the other fragmentation peaks were observed at 511[M+H]⁺, 348[M-162]⁺, 389[M-C₆H₅COO]⁺, 387[M-gentisyl alcohol] and 244 [M-benzoyl alcohol].

The ¹H-NMR spectrum of the compound showed a doublet at δ 3.82 (J=1.2 Hz) for an aromatic proton and the usual ABX spin system of the gentisyl alcohol which was identified by the presence of signals at δ 5.024 (dd, J=2.9 Hz, 8.2 Hz, H-5), 5.028 (d, J=2.4 Hz, H-3), 5.03 (d, J=3.7 Hz, H-6). The two benzoyl residues were observed as ten proton signals in its ¹H-NMR which suggested the presence of two benzoyl residues present in the compound [15]. A broad triplet at δ 3.82 (J=8.3, H-3'') was assigned for one methine group of sugar moiety [16]. The ¹H-NMR and ¹³C-NMR data displayed the resonances that were readily attributed to β -glucose a substituted gentisyl alcohol and two benzoate esters. The downfield shift of C-3'' (+1.3 ppm) and upfield value of C-2'' (-1.7 ppm) were observed by comparison with similar values of salireposide [17], which indicate that the additional benzoyl residual was attached to C-3'' of the glucose moiety [18]. Thus on the basis of above discussion it was identified as **2-[(benzoyloxy)methyl]-4-hydroxyphenyl β -D-glucopyranoside 3-Benzoate**. [Fig. 2]

4. CONCLUSION

It is quite evident that the *Salix babylonica* contains several significant bioactive compounds and some have already shown their therapeutic potential. The present study showed that the roots of *Salix babylonica* is an important bio resource for the extraction and isolation of several metabolites. In future *Salix babylonica* may play a very important role in modern system of medicines.

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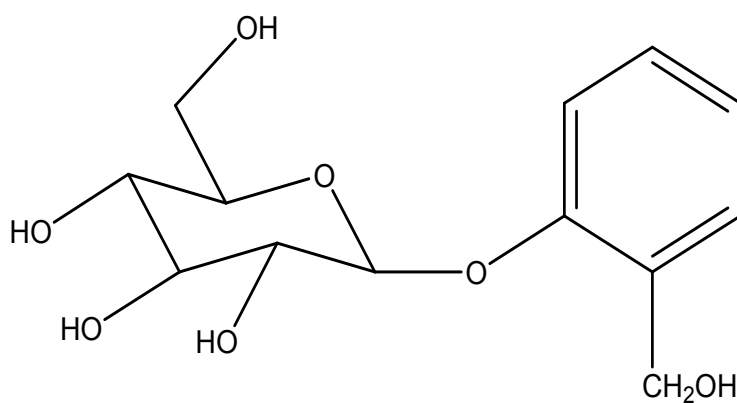


Fig. 1

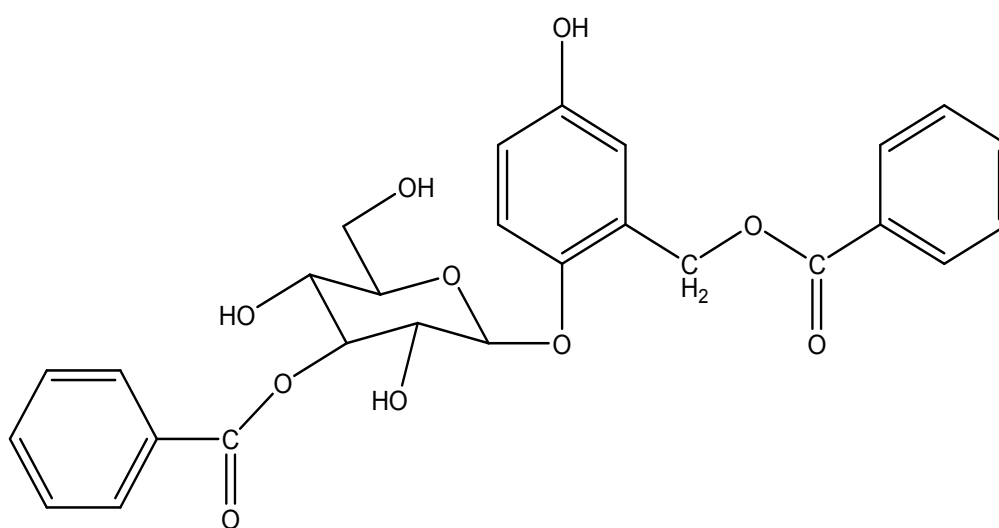


Fig. 2

Table 1

Positions	δ_c ppm	δ_H , ppm (J Hz)
1	132.6	
2	131.6	8.00 (2H,d, J=8.8)
3	117.2	7.37 (2H,d, J=8.8)
4	163.0	
5	117.2	7.37 (2H,d, J=8.8)
6	131.6	8.00 (2H,d, J=8.8)
1'	101.5	5.72 (1H, d, J=7.6)
2'	74.7	4.33-4.38 (3H, m)
3'	77.9	4.33-4.38 (3H, m)
4'	71.2	4.33-4.38 (3H, m)
5'	78.2	4.57 (1H, brd)
6'	62.4	4.40 (1H, brd)
OH		9.00 (1H, s)
CH ₂	60.9	3.60 (2H, s)

Table -2

position	δ_c ppm	δ_H ppm
1	149.6	
2	134.2	
3	116.2	5.028 (d, J=2.4 Hz)
4	153.3	
5	114.4	5.024 (dd, J=2.9, 8.2 Hz)
6	118.6	5.03 (d, J=3.7 Hz)
7	69.1	3.89 (dd, J=6.7, 6.2 Hz)
1'	132.1	
2',6'	131.5	8.13 (dd, J=1.2)
3',5'	120.7	7.16 (brs)
4'	134.2	7.17 (brs)
7'	168.7	
1''	102.4	3.72 (d, J=1.2 Hz)
2''	74.4	4.59 (brs)
3''	78.3	3.82 (brt, J=8.3 Hz)
4''	70.2	3.68 (brt, J=6.9 Hz)
5''	78.4	3.91 (dd, J=2.8, 6.2 Hz)
6''	60.7	3.69 (t, J=7.2 Hz)
1'''	133.6	8.10 (s)
2, ''' 6'''	131.7	3.91 (brs)
3, ''' 5'''	29.6	7.97 (brs)
4'''	134.4	
7'''	167.8	7.98 (brs)

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