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Isolation and Characterization of New Flavonoid Glycoside from the Seeds of Prunus cerasoides

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Abstract
New flavonoid glycoside has been isolated from the seeds of *Prunus cerasoides* and was characterized as 7-O-*β*-D-galactopyranosyl-5-O-methyl naringenin [1].

Keywords: Flavonoid glycoside**,** *Prunus cerasoides,* methyl naringenin.

1. Introduction
Prunus cerasoides (Prunus puddum) belong to the family Rosaceae which is commonly known as Payu, Padam, Padmakha, and Himalayan cherry. Plant wildly growing in sub-Himalayan tracts to montane zone 2400 mtr high, Sikkim, Nepal, Bhutan, Myanmar, West China, and also cultivated in Dhanolti region in Tehri Garhwal (Uttarakhand), India [1, 2, 3] Morphologically the plant is a deciduous tree to 10 m high; bark reddish-brown,exfoliating in thin circular strips. Leaves conduplicate in bud, elliptic or ovate-lanceolate, flowers pinkish-white, 1.5-2.5 cm across, appearing before the leaves in umbellate fascicles, pedicels 0.5-2 cm long. Calyx bell shaped 5 lobed, lobes ovate-acute [1, 2, 3] The stem is bitter acrid, antipyretic, refrigerant, vulnerary; causes flatulence; cures leprosy, hallucination, leucoderma, erysipetals: useful in vomiting, thirst, hiccough, asthma and prevent abortion (Ayurveda) the kernel is used in stone and gravel. The bark contains amygdalin, and the smaller branches are sold in the bazaars as substitutes for hydrocyanic acid in native practice. Braches for walking sticks; bark in psycho medicines leaves as fodder and ripe fruits edible; the juice from the bark applied on body swellings and contusion. Flowers useful source of be-forage plant regarded as sacred, useful in several rituals of locals. The seeds are used for the treatment of stone in the kidney [1, 2].

The stem is reported to be antipyretic, refrigerant and useful in vomiting, thirst, Asthma, leprosy and leucoderma [3]. Different workers [4, 5, 6, 7] have investigated the various parts of the plant and reported the isolation and identification of following compounds from *Prunus cerasoides* (stem bark, leaves, flowers etc.), Tectochrysin, Genistein, Leucocyanadin, Genkwanin, Prunetin, Sakuranetin, 4'-glucoside of genkwanin and naringenin 4'-methyl ether-7-O-β-D-glucoside. In view of the interesting medicinal properties and the fact that a very little work has been reported on the seeds of *Prunus cerasoides* except Luthra [8] *et al.* 1990, prompted us to carry out the detailed phytochemical investigation of seeds.

2. Material and Methods
The melting point is uncorrected The UV spectrums were measured on a Hitachi 320. PerkinElmer motel 202 automatic recording spectrophotometer and Toshinwal manual spectrophotometer. The IR spectra were recorded KBr pellets on Perkin Elmer model 577 and KBr discs (JASCO–IR–810 spectrometer). The ^{1}H – NMR were recorded on Varian EM 360 and were run at 60 and 100 MHz and $^{[13]}$C - NMR spectra at 25.1 MHz (JEOL JNM – MHz and JUM – PX 100 spectrometer) using TMS as internal standard. Mass spectra (70ev, JEOL–TMS – DX 300 spectrometer) were lake with in a direct inlet C, H estimation were came out on Thomas CH – Analyzer 35 Cario Erba – 1106.

2.1. Plant Material
The plant material was collected from Dhanolty, Tehri Garhwal UK (India) in the month of March 2009. The authentification of plant material was made at the Department of Botany, HNB Garhwal University, Campus Badshahithaul, Tehri Garhwal UK, India. A voucher specimen is available at the herbarium of Botany Department.

2.2. Extraction and Isolation

The ethanolic extract was concentrated under reduced pressure to afford a yellowish mass. This solid is subjected to chromatographic resolution over silica gel. The elution was carried out with chloroform, methanol. Eluents were collected in fraction of 80-90 ml.

2.3. Isolation of 7-O-β-D-Galactopyranosyl-5-O-Methyl Naringenin '1'

Fractions No. 56 on removal of solvent furnish a viscous syrupy mass (120 mg) which was purified by acetylation.

2.3.1. Preparation of acetate: The compound '1' (90mg) was treated with acetic anhydride (1ml) and pyridine (2-3 drops) at room temperature to afford an acetate (126mg) m.p. 197-199^0(MeoH). The structure of acetate was established with the help of spectral studies. Analysis Found: C, 58.28; H 5.14%, Calcd. for: $C_{32}H_{34}O_{15}$, C, 58.35, H, 5.20% IR : ν_{max}^{KBr} 1745, 1680, 1610, 1225, 1060, 830, 725 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ ppm 2.05, 2.06, 2.10, 2.11, 2.30, (each 3H, s), 2.8 -2.9 (2H, m), 3.81 (3H, s), 4.24 (2H, br-d, J = 4Hz), 5.00 -5.49 (m, 5H), 6.26(d, 1H, J =2Hz), 6.40(d, 1H, J =2Hz), 7.15(d, 2H, J=9Hz), 7.47 (d, 2H, J =9Hz), ^{13}C-NMR: ($CDCl_3$) δ (ppm) (*cf.* Table-1). MS: m/z 658 (M^+) other peaks observed at 331, 271, 169, 109,

2.3.2. Deacetylation of acetate: The Acetate (30mg) was refluxed for 10 min with 1ml of 10% KOH in MeOH (2ml). The reaction mixture was concentrated and submitted to preparative TLC (solvent $CHCl_3$ MeOH, 7:3) to give 12 mg of deacetylated compound '1' (m.p.142-143 MeOH) TLC (silica gel, R_f = 0.83, solvent system 'L' sprayed with 5% alcoholic $FeCl_3$).

2.3.3. Deacetylated compound '1' Analysis Found: C, 58.85; H, 5.30%**,** Calcd. For: $C_{22}H_{24}O_{10}$, C, 58.92; H,5.35%**,** UV : λ_{max}^{EtOH} 290, 335 nm**,** IR : ν_{max}^{KBr} 3425, 2950, 2850, 1685, 1640, 1550, 1500, 1450, 1360, 1275, 1130, 1050, 996, 825, and 725 cm^{-1}**,** ^{1}H- NMR ($CDCl_3$) δppm 7.81 (d, 2H, J =9 Hz), H-2, and 6'), 6.92 (d, 2H, J =9 Hz, H-3', 5'), 6.82 (d, 1H J = 2.5 Hz, H-8), 6.44 (d, 1H J=2.5 Hz, H-6') 5.20 (s, 1H, J=9 Hz, H-2), 2.80 (s, 1H, J =17 Hz, 3-Heq), 3.30 (d, 1H, J = 2Hz, 3-Hax), 3.85 (s, 3H, -OCH_3), 5.60 (s, 1H, OH exchangable with D_2O), 4.90 (d, 1H J=6.5Hz, anomeric proton of galactose), 3.95 - 4.10 (m, sugar proton), ^{13}C-NMR: (DMSO-d_6) δ ppm (*cf.* Table-1). MS 448 (M^+) 286 (m-sugar M^+ of aglycone, $C_{16}H_{14}O_5$), 167, $C_8H_7O_4$), 120.

Hydrolysis of 7–O–β–D–galactopyranosyl–5–O–methyl naringenin '1'. Compound (1, 25 mg) on enzymatic hydrolysis with almond emulsion at 40 ^{0}C for 24 hrs gave galactose (PC) and aglycone (12 mg). The aglycone (7 mg) was treated with HI to afford a well-known flavanone, naringenin (co-tlc, co-IR, mmp) 5-O-methyl naringenin (aglycone). 5-O-methyl naringenin the aglycone obtained by enzymatic hydrolysis of '1'. Its structure was established with the help of EIMS studies. Acid hydrolysis of H (with 7% H_2SO_4) yielded galactose (Co-PC, Co-IR, mmp) hydrolysis with almond emulsion gave galactose thereby confirming β-configuration of sugar. Analysis Found: C, 67.10, H, 47.01, Calcd for: $C_{16}H_{14}O_5$, C, 67.13; H; 4.69%, MS: m/z 286 (M^+) other peaks observed at 167 and 120.

3. Results and Discussion

3.1. Characterization of '1' as "7-O-β-D-Galactopyranosyl-5-O-Methyl Naringenin

On the basis of elemental analysis and molecular weight determination (M^+448) the molecular formula of compound (m.p. 142 $^{0—}$143 ^{0} homogeneous in TLC) was exhibited as $C_{22}H_{24}O_{10}$. The compound was purified by acetylating of it followed by deacetylation (10% KOH) and was crystallized from MeOH as yellow needles. The compound gave positive test with Molisch reagent but did not reduce fehling's solution. It also responded to characteristic test of flavones (brown color with $FeCl_3$ and cherry red color with Mg/HCl) [9]**.** Acid hydrolysis of compound (with 7% H_2SO_4) gave galactose (co-PC) and an aglycone which gave positive test of Flavanone. This revealed the compound to be a flavonoid- glycoside. The presence of one methoxyl group was determined with the help of Zeisel's method [10]**.** On acetylation, a penta acetyl derivative was obtained there by indicating the presence of five hydroxyl groups on it. The aglycon so obtained was treated with HI to afford the flavanone which was identified as naringenin. The identification of which along with its acetate was established with the help of spectral studies and finally confirmed by comparison with an authentic sample of naringenin (mmp, co-ir, co-tlc.) [11].

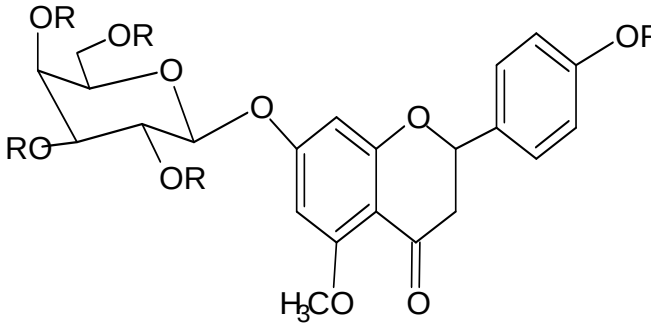
Thus the aglycone should be methyl ether naringenin. The structure of agycone was established as naringenin 5- methyl ether. On the basis of following spectral studies the structure of aglycone was confirmed and consequently that of glycoside was interpreted as naringen –5-methyl ether-7-O-β-D-galactoside. UV (λ_{max}^{EtOH}) absorptions were 290 and 235. There were not shifted by $AlCl_3$ or NaOAC. This confirmed that both the position 5 and 7 are substituted.

The ^{1}H-NMR of the compound and its acetate indicate the presence of a flavanone skeleton [12]. The presence of one aromatic (δ 2.30 ppm) and four alcoholic (δ 2.5, 3.06, 2.10 and 2.11) acetoxyls and an aromatic methoxyl group (δ 3.81) was revealed by the NMR spectrum of acetate. Therefore the compound 1 should be the mono-O-glycosylated, mono-O-methylated naringenin. The chemical shifts for the protons of the B and C-rings of acetate are in good agreement with those of triacetyl naringenin, where as H-6 and H-8 of the compound 1 were observed to be shifted upfield. Accordingly, the OH at C-4' must be unsubstituted[11]. The existence of methoxyl group on ring –A was confirmed by the presence of ions at m/z 286, 167 and 120 in the EIMS of the compound 1[11]. The EIMS of acetate exhibited ions typical of 2,3,4,6- tetra -O-acetyl -β-D-galactoside (m/z 231, 271, 169 and 109) [13]. The chemical shift of sugar moiety in the ^{13}C-NMR of compound 1 and its acetate supported the identification that the attached sugar was galactose [13, 14]. The position at which the methyl group attached was determined as follows. The C-10 (δ 106.5ppm) scarcely shifted on acetylation (δ 107.0ppm) in the ^{13}C-NMR of compound. On the other hand both C-6 and C-8 (δ 93.7 and 95.1) shifted upfield (δ 95.8 ppm) (*cf*. Table-1)**.** Moreover, the chemical shifted of C-10 was consistent with that of 5 methylated flavonoids. Likewise the shift of C-1" was consistent with of 7-O-galactoside [15]. B-Configuration of galactose was confirmed by ^{1}H-NMR (δ 4.90, *J*= 6.5 H_2) and 13C-NMR (δ 100.1ppm). Thus based on the above discussion the structure of aglycone was elucidated as neringenin -5-O-methyl ether and compound 1 as 7- O -*β* -D-galactopyranosyl -5-O-methyl naringenin (reported for the first time from *Prunus cerasoides*).

Table 1: ^{13}C-NMR Assignments in δ ppm

Carbon No.	Compound 1 (DMSO-*d6*)	Compound 1 Acetate (in $CDCl_3$)	Deacetylated Aglycone of 1 (Naringenin) (DMSO-*d6*)	Naringenin triacetate (in $CDCl_3$)
C – 2	78.3(d)	78.7(d)	78.4(d)	79.0(d)
C – 3	44.5(t)	45.5(t)	42.0(t)	45.1(t)
C – 4	192.4(s)	187.4(s)	196.2(s)	188.9(s)
C – 5	159.9(s)	158.8(s)	163.6(s)	151.2(s)
C – 6	93.7(d)	95.8(d)	95.9(s)	110.6(d)
C – 7	165.6(s)	164.5(s)	166.7(s)	156.0(s)
C – 8	95.1(d)	98.8(d)	95.0(d)	109.1(d)
C – 9	165.1(s)	165.4(s)	162.9(s)	163.1(s)
C – 10	106.5(s)	107.0(d)	101.8(s)	111.8(s)
C – 1’	126.0(s)	136.1(s)	128.9(s)	135.6(s)
C – 2’	130.8(d × 2)	127.3(a × 2)	128.2(d × 2)	127.4(d × 2)
C – 3’			115.2(d × 2)	
C – 4’	115.9(d × 2)	121.9(a × 2)	157.8(s)	122.0(d × 2)
C – 5’			115.2(d × 2)	
C – 6’	159.6(s)	150.8(s)	128.2(d ×2)	151.0(s)
O – CH3	115.9(d × 2)	121.9(d × 2)	–	122.0(d × 2)
C – 1”	130.8(d ×2)	127.3(a × 2)	–	127.4(d × 2)
C – 2”	55.5 (q)		–	
C – 3”	100.1(d)	55.7(q)	–	–
C – 4”	73.0(d)	99.3(a)	–	–
C – 5”	76.0(d)	70.3(d)	–	–
C – 6”	68.8(d)	72.6(d)	–	–
Acetyl	77.8(d)	68.5(d)		–
	59.5(t)	72.3(d)		–
	–	62.1(t)		–
	–	170.5(s)		169.2 × 2(s)
	–	170.2(s)		168.0(s)
		169.6(s)		21.1 × 3(q)
		169.4(s)		
		169.3(s)		
		21.1(q)		
		20.8(q)		
		20.6×3 (q × 3)		

Not: - s = singlet, d = doublet, t = triplet q = quartet



Compound 1: R=H R = AC (Acetate of ‘1’)

4. Conclusion

The investigated species *(Prunus cerasoides)* was rich in flavonoids and their glycosides; therefore plant seeds were taken for present study and find flavanone from Dhanolti region and compound report for first time. Bioactivity of the compound is under progress and benefited for further research.

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