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Short Communication

Chemical Constituents of *Prunus persica* Stem Bark

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Abstract

Chemical examination of methanolic extract of *Prunus persica*, stem bark, led to isolation of Flavon 3', 4', dihydroxy 6 methoxy 7-O-α -L-rhamnopyranoside (1), Prunetin-5-O-β-D-glucopyranoside (2), with previously known compounds β-Sitosterol and Querceitin. Compounds 1 and 2 were isolated for the first time from this plant. The structure of compounds were elucidated with the help of chemical and spectral studies.

Keywords: Prunus persica; Methanolic extract; Flavonoidal glycoside.

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1. Introduction

The green plants are the storehouses of many chemical components. They have a special capability of converting simpler inorganic compounds into complex organic compounds, which are used for several metabolic activities called metabolites. Metabolites of plants are grouped into two categories namely primary metabolites and secondary metabolites. The primary metabolites are used for the growth of plants and also for their survival. But secondary metabolites do not play a considerable role in the growth of the plant. As these compounds receive secondary importance in plant growth they are named as secondary metabolites. On the contrary they are the principal components that play an important role against diseases and disorders in both plants and human beings. The primary metabolites included carbohydrate, proteins, enzymes, lipids, vitamins and growth hormones of plant. Secondary metabolites are the substances, which are produced by plants as defense chemicals. It includes alkaloids, flavonoids, essential oils, phenols, terpenes etc [1].

Prunus persica (Aaru) belongs to the family *Rosaceae* is a deciduous tree up to 10 m high. Bark gray or ashy or serrate is acuminate glabrous. Flowers are pinkish white sessile

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or short, pedicelled. These plants are commonly cultivated for edible fruits in sub-Himalayan region at a height of upto 2400 m [2-3]. The chemical constituents of the herb included the cyanogenic glycosides, amygdalin and prunasin as major components along with glycerides, sterols, and emulsin [4]. Recently, glycosides from this plant seeds have been reported for the anti-tumor activity promoting Epstein-Bar virus activity in early antigen-infected lymphoblastoid cells [5]. Amygdalin is also abundant in the seeds of bitter almond and apricots of the *Prunus* genus, and other rosaceous plants. Amygdalin extracted from *Persicae semen* was studied for anticancer activity [6]. An alkaloid persicaside with anti inflammatory activity was isolated from its seeds as well [7]. A large variety of compounds alkaloid, terpenoid and flavonoid were isolated from the genus [8-15]. The present paper deals with the isolation and structure elucidation of a flavonoidal glycoside 1 and 2 and from the methanolic extract of stem bark of *Prunus persica*.

2. Experimental

2.1. *Materials and Methods*

Melting points were recorded on a Perfit melting point apparatus. UV spectras were measured on a Perkin-Elmer Lambda-25 spectrophotometer in methanol. IR spectra recorded on a Perkin-Elmer Spectrum RX1 FT-IR spectrometer (KBr discs). NMR spectra were obtained on Bruker Avance 300 and 500 spectrometers (300 MHz for ^{1}H and 125 MHz for ^{13}C using $CDCl_3$ as solvent and TMS as internal standard). MS were recorded on Atmospheric Pressure Chemical Ionization Mass Spectrometer (APCIMS). Column chromatography was performed on silica gel (Merck 60-120 mesh, 15 × 100 cm). TLC was carried out on silica gel (Merck 10-40 μ) precoated plates, spots were visualized by spraying with 7% H_2SO_4.

2.2. *Plant material*

Stem bark of *Prunus persica* were collected from Singoli Tehri Garhwal Uttarakhand, India and identified from the Plant Identification Laboratory, Department of Botany, H. N. B. Garhwal University Srinagar. A voucher specimen (GUH 8388) was deposited in the Department for future records.

2.3. *Extraction and isolation*

The air dried stem bark of *Prunus persica* (3 kg) were powdered and extracted exhaustively with methanol (4 times) to yield a red extract, which was concentrated under reduced pressure and defatted with n-hexane. The extract (200 g) was pre-adsorbed with silica gel and applied on the top of a column prepared by silica gel (600 g) in $CHCl_3$. The elution was first started with $CHCl_3$ and then $CHCl_3$ with increasing amounts of MeOH.

Elution with $CHCl_3$:MeOH (92:8) afforded compound 1 and (90:10) afforded compound 2.

2.4. *Compound 1*

White crystalline powder (MeOH), M.P. 312-313°C, Molecular formula $C_{22}H_{22}O_{10}$, Molecular weight 446 amu, APCIMS (m/z) 446$[M]^+$, 447$[M+H]^+$, 469$[M+Na]^+$, 485$[M+K]^+$ 301$[M+H-146]^+$, 429$[M+H-H_2O]^+$, 279, 249, 230, 219,149, ^1H and ^{13}C NMR (DMSO) data are given in Table 1.

2.5. *Compound 2*

It was crystallised from methanol as crystalline solid, M.P. 237-239°C, Molecular formula $C_{22}H_{21}O_{10}$, Molecular weight 445 amu, UV (λ_{max}^{MeOH}) nm 363, 327(Sh), 259(+$AlCl_3$-HCl), 259(+NaOAc), 259 (+NaOAc-H_3BO_3), 259(+NaOAc), 280, APCIMS (m/z) 445$[M]^+$, 469$[M+H+Na]^+$, 490$[M-H+2Na]^+$, 372, 282$[M-H-162]^+$, 260, 230. ^1H and ^{13}C NMR ($CDCl_3$) data are given in Table 2.

3. Results and Discussions

3.1. *Compound 1*

It was isolated as yellow crystals from methanol. The APCIMS spectrum of compound 1 showed molecular weight of 446 amu, which corresponds to the molecular formula $C_{22}H_{22}O_{10}$. It gave positive test with $FeCl_3$, Mg/HCl and Molish test thereby showed it to be a flavonoid glycoside. The UV spectrum of the compound showed absorption band at 270, 276, 428 nm and IR absorption band appeared at 3410, 1650, 1525, 1430 cm^{-1} which were characteristic for flavonoid glycoside. The ^1H NMR spectrum of compound 1 displayed a typical signal of flavonoid, the presence of two doublets at δ 6.89 and δ 6.66 with coupling constant 7.0 and 5.5 Hz were assigned for H-3 and H-5'. The three singlet at δ 7.3, δ 7.7 and δ 6.95 were characteristic for unsubstituted H-2', H-8, H-5. A sharp singlet at δ 3.09 was assigned for aromatic methoxyl position at C-6, other singlet at δ 1.27 was assigned for rhamnose methyl group. The position of anomeric proton at δ 5.95 (s, 1H) indicated the α configuration of the rhamnose sugar. The ^{13}C NMR spectrum of the compound 1 displayed twenty two carbons, peak at 168.0 was assigned for carbonyl carbon atom where as the peaks at 149.0, 129.5, 134.1 and 110.1 were assigned for oxygenated substitution at C-6, C-4', C-3' and C-7 positions. The down field value of 110.1 of C-7 showed glycosidation at this point. The up field signal at 17.5 assigned for rhamnose methyl, whereas signal at 54.3 was depicted for methoxy function. On the basis

of above spectral data the compound was identified as flavon 3', 4', dihydroxy 6 methoxy 7-O-α -L-rhamnopyranoside (Fig. 1). The ^{1}H-NMR and ^{13}C-NMR, data of compound 1 are given in Table 1.

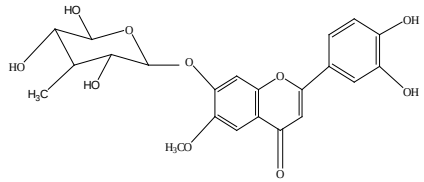

Fig. 1. Structure of compound 1: flavon 3', 4', dihydroxy 6 methoxy 7-O-α -L-rhamnopyranoside.

Table 1. ^{1}H-NMR and ^{13}C-NMR, data of compound 1.

Positions	δ_C ppm	δ_H ppm, J(Hz)	Positions	δ_C ppm	δ_H ppm, J(Hz)
2	149		5’	113.9	6.66 (d, J=5.5)
3	118.9	6.89(d, J=7.0)	6’	111.8	
4	168		1’’	103.2	5.95 (1H, s)
4a	110.1		2’’	86.1	4.73
5	137.8	6.95 (s)	3’’	71.9	4.71
6	149		4’’	73.7	
7	110.1		5’’	71.6	
8	86.3	7.7 (s)	6’’	55.6	
1’	129.7		OCH_3	54.3	3.09(3H, s)
2’	118.7	7.3 (s)	CH_3	17.5	(3H, s)
3’	134.1				
4’	129.5				

3.2. *Compound 2*

It was crystallized from methanol as crystalline solid. Molecular ion peak was observed at m/z 445$[M]^+$ and the other fragment peaks were obtained at m/z 469$[M+H+Na]^+$, 490$[M-H+2Na]^+$, 272. The peak at m/z 282$[M-H-162]^+$ arose by loss of one hexose unit from molecular ion peak. The UV spectrum of the compound 2 showed a prominent maximum at 259 indicating isoflavonoid nucleus which was supported by a ^{13}C chemical shift of 148.3 for a methylene carbon which corresponds to C-2 of an isoflavone and excluded the isomeric flavone structure [16]. Moreover the H-2 chemical shift value of δ 8.15 indicated its isoflavone nature. The glycosidic nature of the compound 2 and its sugar moiety was proved by hydrolysis followed by paper chromatography. The aglycon was identified as prunetin and sugar as glucose. The presence of a distinct bathochromic shift

(12 nm) on addition of $AlCl_3$-HCl to its aglycone and its absence in the UV spectrum of the respective glycoside indicated that the sugar was attached to C-5 OH. The ^1H and ^{13}C chemical shift as well as ^1H-^1H coupling constant confirmed that the sugar was glucose and it has β configuration (J=6.0Hz). The sides of the linkage of the two substituents Me and β-D-glucosyl were ascertained by two NOE difference experiments on irradiation of the anomeric proton (H-1"). δ 5.05 signal enhancement were observed for one aromatic proton only (H-6) proving that the sugar is attached to that C-5 OH. The appearance of C-3"/5" responses provided a further argument of glucosyl grouping. The downfield signal at 168.1 (C-4) in its ^{13}C NMR spectrum of compound 2 suggested the presence of carbonyl functional group whereas the anomeric carbon 106.3 (C-1") and methoxyl 54.5 (OMe) were resonated in its ^{13}C NMR spectrum which confirmed the presence of β linked sugar and methoxyl group at C-5 and C-7 positions of the compound. Irridiation of methoxy

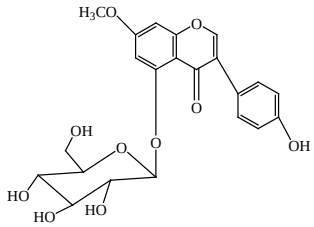

Fig. 2. Structure of compound 2: prunetin-5-O-β-D-glucopyranoside

Table 2. ^1H-NMR and ^{13}C-NMR, data of compound 2.

Positions	δ_C ppm	δ_H ppm, J(Hz)	Positions	δ_C ppm	δ_H ppm, J(Hz)
2	148.3	8.15 (s)	1"	106.3	5.05 (d, J=6.4)
3	127.6		2"	101.39	3.84 (dd, J=6.0, 3.0)
4	168.1		3"	101.3	3.33 (dd, J=5.0, 5.0)
5	147.5		4"	86.2	3.38 (dd, J=9.0, 9.0)
6	106.3	6.82 (d, J=4.0)	5"	71.6	
7	148.3		6"	55.5	3.31 (dd, J=4.0, 2.0)
8	107.9	6.20 (d, J=3.0)	6"		3.58 (dd, J=7.8, 6.8)
9	147.5		OCH_3	54.5	3.95 (s)
10	114.1				
1'	122.3				
2'	129.7	5.97 (m)			
3'	118.9	6.87 (m)			
4'	142.1				
5'	118.9	5.44 (d, J=6.0)			
6'	129.7				

proton however induced NOE of both the H-6 and H-8 signals. Thus this substituent is positioned between them i.e. at C-7 OH, the NOE on H-6 is clearly smaller than that on H-8 therefore the methyl group is directed preferably towards the H-8 atom due to its steric interfere with the bulky sugar moiety. Thus on the basis of above studies compound 2 was identified as prunetin-5-O-β-D-glucopyranoside (Fig. 2). The ^{1}H-NMR and ^{13}C-NMR, data of compound 2 are given in Table 2.

4. Conclusion

It is quite evident that the *Prunus persica* contains several important bioactive compounds and some have already shown their therapeutic potential. The present study shows that the stem bark of *Prunus persica* is an important bio resource for the extraction and isolation of flavonoids. Thus in future *Prunus persica* may play a very important role in modern system of medicines.

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