

CHEMICAL EXAMINATION AND ANTI INFLAMMATORY ACTIVITY OF *PRUNUS PERSICA* STEAM BARK¹RAKESH RATURI*, ²S.C. SATI, ¹HARPREET SINGH, ²M. D. SATI, ¹P. BAHUGUNA AND ¹P. P. BADONI¹Department of Chemistry, HNB Garhwal Central University Campus Pauri, ²Srinagar, Garhwal, India. Email: raaakeshhh@gmail.com

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ABSTRACT

Chemical examination of methanolic extract of *Prunus persica* steam bark, led to isolation of Acetophenone 6-hydroxy 4-methoxy 2-O- β -D-glucopyranoside (1), Crysophenol 8-O- β -D-glucopyranoside (2), along with β -Sitosterol and Quercetin. The structure of compounds 1 & 2 were elucidated with the help of chemical and spectral studies. The methanolic extract of plant was also evaluated for anti inflammatory activity against the wistar species of rats.

Keywords: *Prunus persica*, Carraginine, Inflammatory activity

INTRODUCTION

Over the last few years researches have aimed at identifying and validating plant derived substances for treatment of various disease. Interestingly, it is estimated that more than 25% of modern medicines are directly or indirectly derived from plant¹⁻³. In this context, it is worth mentioning that Indian medicinal plants are considered a vast source of several pharmacologically active principals and compounds and that are commonly used in home remedies against multiple ailments⁴⁻⁶. *Prunus persica* (Aaru) belongs to the family *Rosaceae* is a deciduous tree up to 10 m. high. Bark gray or ashy or serrate acuminate glabrous. Flowers pinkish white sessile or short, pedicelled. Commonly cultivate for edible fruits from sub-Himalayan region up to 2400 m. ⁷⁻⁸. The chemical constituents of the herb include the cyanogenic glycosides, amygdalin and prunasin as major components along with glycerides, sterols, and emulsion ⁹. Recently, glycosides from this plant seeds have been reported for the anti-tumor activity promoting Epstein-Bar virus activity in early antigen-infected lymphoblastoid cells¹⁰. 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 activity¹¹. An alkaloid persicaside with anti inflammatory activity was isolated from its seeds¹². The present paper deals with the chemical investigation and anti inflammatory activity of Methanolic extract of *Prunus persica* stem bark.

MATERIALS AND METHODS

Collection of Plant material

Stem bark of *Prunus persica* were collected in October 2007 from, Singoli Tehri Garhwal Uttarakhand India and identified from the Plant Identification Laboratory, Department of Botany, and H. N. B. Garhwal University Srinagar. A voucher specimen (GUH 8388) was deposited in the Department.

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 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 β -Sitosterol and Quercetin.

RESULTS AND DISCUSSION

Characterization of Compound 1

M. P. 202-203°C,

Molecular formula C₁₅H₂₀O₉,

Molecular weight 344 amu

IR (λ_{\max} KBr) cm⁻¹ 3240, 3040, 1895, 1660, 1450, 1250, 1190, 850, APCIMS(m/z) 344[M]⁺, 342[M-2H]⁺, 345[M+H]⁺, 356[M+H+Na]⁺, 180 [M-2H- 162]⁺, 121, 102,¹H -NMR (DMSO) δ ppm6.71 (d, 1H, J=5Hz, H-3), 6.76 (d, 1H, J=5Hz, H-5), 7.92 (s, 1H, 6-OH), 5.14 (d, 1H, J=6.2Hz, anomeric of glucose), 3.60 (s, 3H, OCH₃), 2.68 (s, 3H, O=C-CH₃), 4.07-4.92 (sugar multiplet)¹³C-NMR (CDCl₃) δ ppm

Aglycone

109.9 (C-1), 104.2(C-2), 82.6(C-3), 166.9(C-4), 92.6(C-5), 167.4(C-6), 61.1(C-7), 33.5 (C-8), 169.0 (C=O)

Glycone

104.2 (C-1'), 74.7 (C-2'), 78.2 (C-3'), 72.1 (C-4'), 82.6 (C-5'), 62.5 (C-6')

Compound 1 was crystallized from methanol as white amorphous powder. The IR spectrum of the compound displayed a characteristic absorption band of OH group at 3110 cm⁻¹ and 1630 cm⁻¹ for carbonyl group. The IR spectrum also displayed the absorption band for other at 1250 and 1070cm⁻¹, its elemental analysis corresponded to molecular formula C₁₅H₂₀O₉ which was confirmed by its molecular ion peak at m/z 345 [M+H]⁺. The other fragments ion peak were absorbed at m/z 342[M]⁺, 356[M+H+Na]⁺, 180[M-2H+162]⁺. The ¹H NMR spectrum of the compound displayed a singlet of 2H at δ 6.57 indicating that compound is symmetrically tetra substituted aromatic compound and integrated broad signal at δ 7.92 was assigned for phenolic group attached to the C-2 and C-6 of the ring. The presence of OH group at C-2 and C-6 was further confirmed by presence of one signal at δ 167.4 in ¹³C NMR spectrum. The ¹H NMR spectrum also showed two singlets for three protons each at δ 3.60 and δ 2.68 indicating the presence of methoxyl (OCH₃) and acetyl (COCH₃) group present in the compound which was further confirmed by ¹³C NMR chemical shift of carbonyl carbon atom at 166.9. A methyl carbon attached to C=O at 33.5 and methyl carbon resonate at 61.1, methoxyl group was found to be attached at C-4 carbon atom and acetyl group at C-1 carbon atom as deduced from the ¹³C NMR shift of C-1 at 109.9 and C-4 at 166.9. The β -linkage of sugar was ascertained by the coupling constant (J=6.2Hz, H-1' of anomeric proton). The other sugar protons were found to appear in between δ 4.07-4.92 as a multiplet in ¹³C carbon NMR spectrum. Fifteen carbon atom are resonated at 109.9(C-1), 104.2(C-2), 82.6(C-3), 166.9(C-4), 92.6(C-5), 167.4 (C-6), 33.5(CH₃). The down field signal at 166.9 in ¹³C NMR spectrum of compound indicate the presence of α , β unsaturated carbonyl group present in the compound. The molecular ion peak at m/z 180[M-2H-162]⁺ shows the loss of one hexosyl unit from the molecular ion peak. Thus the mass loss of 162 amu from molecular ion peak showed the presence of hexose sugar present in the molecule. Thus on the basis

of above spectral studies the compound 1 was identified as Acetophenone 6-hydroxy 4-methoxy 2-O-β-D-glucopyranoside [Fig. 1].

Characterization of Compound 2

It was obtained as yellow crystalline solid from ethyl acetate,

M. P. 160-162°C,

Molecular formula C₂₁H₁₉O₉,

Molecular weight 415 amu,

FABMS 415[M]⁺, 416[M+H]⁺, 439[M+H+Na]⁺, 254[M+H-162]⁺,

IR (λ_{max}^{KBr}) cm⁻¹ 3400, 2900, 1630, 834 cm⁻¹

UV (λ_{max}^{MeOH}) nm 225, 290, 420 nm,

¹H and ¹³C NMR (CDCl₃) data are given in [Table 2]

¹H-NMR (DMSO) δppm

7.26 (brs, H-2), 2.5 (s, 3H, H-3), 7.55 (brs, H-4), 7.95 (m, H-5), 7.8 (m, H-7), 5.30 (d, 1H, J=8.0, Anomeric of galactose)

¹³C-NMR (CDCl₃) δppm

Aglycone

158.2 (C-1), 124.0 (C-2), 147.6 (C-3), 123.0 (C-4), 119.3 (C-5), 134.3 (C-6), 114.2 (C-7), 161.6 (C-8), 187.3 (C-9), 181.4 (C-10), 136.0 (C-11), 120.9 (C-12), 131.9 (C-13), 21.5 (Ar-Me)

Glycone

101.3 (C-1'), 73.5 (C-2'), 77.5 (C-3'), 69.9 (C-4'), 76.4 (C-5'), 61.0 (C-6')

Compound 2 gave positive test for anthraquinone. The molecular mass of compound 2 was deduced from its FABMS, the molecular ion peak appeared at m/z 415[M]⁺, 416[M+H]⁺, 439[M+H+Na]⁺, 254[M+H-162]⁺. The peak at 254 [M+H-162]⁺ in its FABMS arose by the loss of hexoxyl (glactosyl) unit from molecular ion peak. On acidic hydrolysis compound afforded an aglycon M.P. 195-196°C identified as crysophenol by MMP and Co-TLC the neutralized hydroxylate on paper chromatography showed the presence the galactose (authentic sample run in parallel). The ¹H NMR spectrum of compound showed a sharp singlet at δ 2.5 for aromatic methyl group, two broad singlets at δ 7.26 and δ 7.55 were assigned for H-2 and H-4 position. Two multiplets at δ 7.8 and δ 7.95 were assignable for H-7 and H-5 position. The anomeric proton for galactose was absorbed at δ 5.3 (d, J= 8.0Hz) in its ¹H NMR spectrum. The ¹³C NMR spectrum of compound showed the fourteen resonance lines for fourteen carbon atoms, of which the anomeric methyl group resonate at 21.5 in order to look into the structure of compound it was methylated by Hakomories method. The aglycone obtained by acidic hydrolysis above methylated product was identified as 8-hydroxy 1-methoxy 3-methyl anthraquinone. This established the attachment the sugar moiety at position 8. The hydrolysate on paper chromatography showed the 2,3,4,6 tetra-O-methyl-D-galactose. The type of linkage was assigned by ¹H NMR and ¹³C NMR spectrum. Thus on the basis of above studies compound 2 was identified as Crysophenol 8-O-β-D-galactopyranoside [Fig. 2]

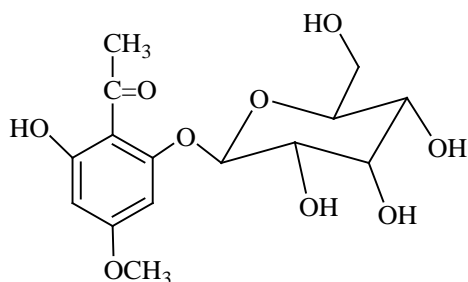


Fig. 1

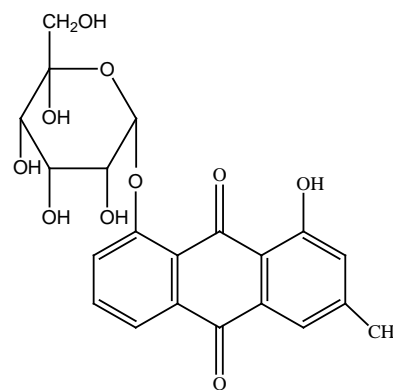


Fig. 2

Structure of compound 1 and 2

Anti inflammatory activity

Animals

Male wistar rats (130-160g) kept the animal house of the IIM Jammu. The animals were housed under standard environmental conditions. All experiment was carried out after getting the approval from the committee for the purpose of control and supervision of experimental animals (CPCSEA) having the registration number is 67/CPCSEA/99.

The anti-inflammatory activity of plant extract was evaluated by carrageenin-induced paw edema method in wistar specie of rats. In carrageenin-induced paw edema model. The plant extract at dose 250mg/kg caused inhibition of paw edema by 4.76 after 4th hours of carageenin administration (Table 3). 1st, 2nd and 3rd hours results were not favorable so we take only 4th hour result. The carrageenin-induced paw edema in rats is believed to be biphasic¹³

Table 3: Anti-inflammatory activity of *Prunus persica* stems bark

Treatment	Edema volume (ml)* 4 th hour	% inhibition
Control	1.26 ± 0.120	-
PP (250/kg)	1.2±0.173	4.97
Ibuprofen (5mg/kg)	0.76 ± 0.066	39.68

* value are mean ± SE, n=3, P>0.01, PP- P. persica extract

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