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Phytochemical analysis of *Tamrix Gallica*

D.P.Pandey[1]*, K.B. Karnatak[1], R.P. Singh[1], M. A. Rather[1], R. K. Bachheti[2] and Sumer Chand[3]

[1]Department of Chemistry, Govt. P. G. College, Uttarkashi-249 193, Uttarakhand, India.

[2]Research and Development Lab, Graphic Era University, Deharadun, Uttarakhand, India.

[3]Systematic Botany Division, FRI, Dehradun, Uttarakhand, India.

Corres. author : pandeydp_123@rediffmail.com,Fax: 01374-222148

Abstract: (E)-Ferulaldehyde, β-Sitosterol-β-D-glucoside, Kaempferol-7-O-methyl ether have been isolated from the whole plant of *Tamrix Gallica*. The concerted use of IR, UV, NMR spectroscopy and chemical methods allowed the identification of these compounds.
Key words: *Tamrix Gallica,* (E)-Ferulaldehyde, β-Sitosterol-β-D-glucoside, Kaempferol-7-O-methyl ether.

INTRODUCTION

Tamrix Gallica Linn (Tamaricaceae) is a large genus of graceful shrubs distributed in the temperature regions of Asia. The species Tamarix. Commonly known as Tamariskis, prefer alluvial soil, but grow on saline and alkaline soil also. It is in flower from June to August. The flowers are hermaphtrodite and are pollinated by bees. The present investigation deals with the isolation and identification of (E)-Ferulaldehyde, β-Sitosterol-β-D-glucoside , Kaempferol-7-O-methyl ether,. and have been isolated from the whole plant of *Tamrix Gallica*.

MATERIAL AND METHOD

Plant Material

Tamrix Gallica were collected from Dehradun, in Feb 2008. The plant species were identified by Dr. Sumer chand, Systematic Botany Division, FRI, Dehradun, Uttarakhand. The voucher specimen (Hr. no. 59) was deposited in the herbarium of Department of Botany, R.C.U. Govt. P. G. College, Uttarkashi, Uttarakhand.

Extraction and Isolation

The air-dried and powdered plant part of *Tamarix gallica* (2.0 kg) was extracted with light petroleum ether (60-80^0), that was concentrated under reduced pressure to give petroleum extract (4.5 g). The petroleum extract (4.5 g) was column chromatographed over Si-gel using gradient elution with C_6-H_6-EtOAc (10:0→9:1) afforded compound 1 (320 mg).

The petroleum free mass was extracted with 60% ethanol. The ethanol extract was concentrated under reduced pressure and a suspension of the residue was made with water. The residue was partitioned with $CHCl_3$:H_2O (6:4) in a separatory funnel. The chloroform layer was separated out and concentrated under reduced pressure to give $CHCl_3$ extract (8.35 g). The $CHCl_3$ extract (7.5 g) was subjected to CC over Si-gel using gradient elution with C_6H_6-EtOAc (10:0→9:1) afforded various fractions having mixture of compounds. The C_6H_6-EtOAc (9:1) fraction (5.0 g) was subjected to CC over Si-gel using gradient elution with C_6H_6-EtOAc (98:2→9:1) afforded compound 2 (170 mg). The fraction obtained with C_6H_6-EtOAc

(9:1) was subjected to CC over Si-gel eluted with C_6H_6-EtOAc (5:5) afforded compound 3 (200 mg).

Compound (1): pale yellow liquid ; Elemental Analysis: C=67.38%, H=5.55%; (calc. $C_{10}H_{10}O_3$); Molecular weight 178. FAB^+-MS : m/z 179 $[M+H]^+$, 177, 167, 149, 137, 135, 107, 91, 65, etc. IR (V_{max}^{nujol}) : cm^{-1} 3250, 2825, 2717, 1660, 1610, 1590, 1500, 1460, 1230, 1050, 760, 715, etc. UV (λ_{max}, MeOH): nm 240, 255, 305, and 340. ^{1}H-NMR: (300 MHz, $CDCl_3$): δ 7.38 (1H, *d*, *J* = 2.0 Hz, H-2), 6.83 (1H, *d*, *J* = 7.0 Hz, H-5), 7.18 (1H, *dd*, *J* = 2.0 & 7.0 Hz, H-6), 7.60 (1H, *d*, *J* = 16.0 Hz, H-7), 6.61 (1H, *dd*, *J* = 16.0 & 8.0 Hz, H-8), 9.61 (1H, *d*, *J* = 8 Hz, H-9), 3.81 (3H, *s*, OMe). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 125.71 (C-1), 111.48 (C-2), 150.13 (C-3), 147.38 (C-4), 115.64 (C-5), 123.96 (C-6), 153.98 (C-7), 115.33 (C-8), 194.63 (C-9), 55.74 (-OMe).

Compound (2) : white crystals, M.P. 289-291°C. (MeOH); $[\alpha]_D^{25}$: -39 (c=0.9, MeOH). Elemental Analysis: C=72.68%, H=10.2%, (calc.); Molecular weight 576. IR (ν_{max}KBr : cm^{-1} 3400, 2910, 1642, 878 and 780.

Compound (3): yellow needles, M.P. 226-227°C. (MeOH); Elemental Analysis C=63.98%, H=3.97%; (calc. $C_{16}H_{12}O_6$); Molecular weight 300. IR (V_{max}^{KBr}) : cm^{-1} 3470, 3260, 1640, 1608, 1500, 1420, 1350, 1280, 770, 720, etc. UV (λ_{max}, MeOH): nm 268, 320, 364, (+$AlCl_3$) 274, 305, 344. ^{1}H-NMR: (300 MHz, CD_3OD): δ 6.23 (1H, *d*, *J* = 1.5 Hz, H-6), 6.49 (1H, *d*, *J* = 1.5 Hz, H-8), 6.87 (2H, *dd*, *J* = 7.8 Hz, H-3',5'), 8.07 (2H, *d*, *J* = 7.8 Hz, H-2',6'), 3.83 (3H, *s*, -OMe). ^{13}C-NMR (75 MHz, CD_3OD): δ 146.97 (C-2), 136.73 (C-3), 177.33 (C-4), 162.19 (C-5), 98.58 (C-6), 167.07 (C-7), 92.73 (C-8), 158.15 (C-9), 105.46 (C-10), 123.65 (C-1'), 130.75 (C-2',6'), 116.39 (C-3',5'), 160.83 (C-4'), 56.47 (-OMe).

Acid hydrolysis of Compound 3: Compound 3 (10mg) was dissolved in 5% H_2SO_4 and refluxed on water bath for 3 hrs. The reaction mixture was cooled and poured on crushed ice and stand for 30 min. The precipitate was purified by re-crystallization from MeOH. The aglycone was identified as p-hydroxybenzoic acid (compound 1) by comparison with authentic sample and the sugar was identified as D-glucose by paper chromatography.

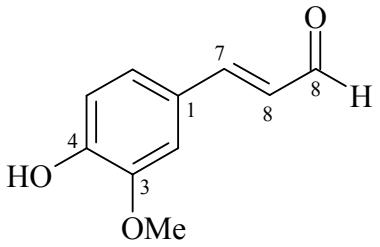
RESULTS AND DISCUSSION

COMPOUND: 1

The elemental analysis corresponded to molecular formula $C_{10}H_{10}O_3$ which was substantiated by the presence of molecular ion peak at m/z 179 in FAB positive ion mass spectrum. The IR spectrum displayed characteristic absorption band at 1660 and 1610 cm^{-1} indicating presence of α,β-unsaturated carbonyl group. The UV spectrum displayed absorption band at 240, 255, 305, and 340 nm which was very similar to ferulaldehyde [1].

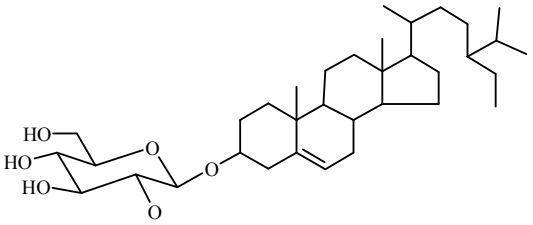
The ^{1}H-NMR spectrum displayed presence of a trisubstituted aromatic ring and a -CH=CH-CHO structuralmoiety in the molecucle. In the aromatic region three signals, each integrated for one proton, appeared at δ 7.38 (*d*, *J* = 2.0 Hz), 6.83 (*d*, *J* = 7.0 Hz), and 7.18 (*dd*, *J* = 2.0 & 7.0 Hz), was assigned for H-2, H-5 and H-6 of 1,3,4-trisubstituted aromatic ring. The tri-substituted aromatic ring was further confirmed by ^{13}C-NMR spectrum, which displayed signals for substituted carbons at δ 125.71 (C-1), 150.13 (C-3), 147.38 (C-4). The downfield shifts of C-3 and C-4 in comparison to C-1 indicated that these carbons bears oxygen functions. The ^{1}H- and ^{13}C-NMR spectrum displayed presence of methoxyl group at δ 3.81 and δ 55.73 ppm, respectively. In addition to these, the ^{1}H-NMR spectrum displayed a doublet at δ 7.60 (*J* = 16.0 Hz, H-7), a double doublet at δ 6.61 (*J* = 16.0 & 8.0 Hz, H-8), and a doublet at δ 9.61 (*J* = 8 Hz, H-9), each for one proton indicated the presence of presence of olifinic double bond substituted with aldehyde group (-CH=CH-CHO). The large value fo coupling constant showed the trans configuration of double bond. The presence of -CH=CH-CHO group was determined by ^{13}C-NMR chemical shifts of olifinic carbon at δ 153.98 (C-7), and 115.33 (C-8), and an aldehyde group at δ 194.63 (C-9) [2,3].

On the basis of these spectral evidences compound 2 was characterized as (E)-feruldehyde; (E)-4-hydroxy-3-methoxy-cinnamaldehyde. The identity of the compound was further confirmed by comparison of spectral data with the reported data [4].



COMPOUND: 2 Hydrolysis of Compound 2: Hydrolysis of compound 2 was carried out by refluxing 5 mg of the compound with 5% H_2SO_4 for 3 hr on water bath. The contents were cooled and extracted with chloroform where a crystalline compound as white needles was obtained; it was identified as β-sitosterol. The sugar was identified by PC as glucose. Compound 2 gave positive Molisch's test and did not reduce Fehling solution. It also

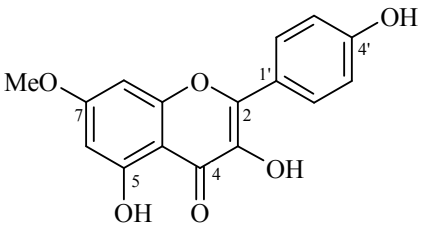
responded to LB test, characteristic for sterols and positive TNM test for unsaturation. The IR spectrum showed characteristic absorption at 3400 (OH stretching) and 1642 (C=C stretching),878 and 780 cm^{-1} (of glycoside) [5]. On acid hydrolysis compound 2 afforded an aglycone (M.P. 135-136°C), identified as β-sitosterol by co-TLC, co-IR and MMP with an authentic sample. The sugar was identified as glucose by co-PC with an authentic sample. On the basis of above observations compound 2 was identified as β-sitosterol-β-D-glycoside. It was further confirmed by co-TLC and co-lR and MMP with an authentic sample.



COMPOUND: 3

Elemental analysis corresponded to the molecular formula $C_{16}H_{12}O_6$. It gave olive green colour with $FeCl_3$ and positive Shinoda test, which indicate the presence of flavonoidal skeleton [6]. Its IR spectrum displayed two absorption band at 3470 and 3260 cm^{-1} for chelated and non-chelated OH groups respectively. The IR spectrum also showed absorption bands at 1640 and 1608 cm^{-1} indicated presence of α,β-unsaturated carbonyl group (-C=O) and at 1500, 1420 cm^{-1} for stretching of ether function.

The ^{1}H-NMR and ^{13}C-NMR data of compound 3 indicated the flavonoid skeleton of the molecule [7, 8]. In aromatic region of ^{1}H-NMR spectrum, two *meta*-



coupled doublet (*J*=1.5 Hz) each for 1H at δ 6.23 (H-6) and 6.49 (H-8) and two *ortho*-coupled A_2B_2-type doublet (*J*=7.8 Hz) at δ 6.87 (H-13, 15) and 8.06 (H-12, 16) suggested the presence of a *tetra*-substituted and a 1,4-di-substituted phenyl ring. The later ring was further confirmed to be *p*-hydroxyphenyl system from the ^{13}C-chemical shift of the carbon signals at δ 130.75 (C-2',6') and δ 116.39 (C-3',5'), which fairly corresponded with those of hydrogen bearing carbons of *p*-cresol (δ 115.3, 130.2) [9]. In aliphatic region the ^{1}H-NMR spectrum displayed an integrated singlet for 3H at δ 3.83 was assigned for -OMe group attached at C-7 position, which was confirmed by downfield ^{13}C-chemical shift of C-7 at δ 167.0. The ^{13}C-NMR spectrum also showed the presence of C=O group at δ 177.33, a benzylic carbon (C-2) at δ 146.97 and oxygen bonded ethylenic carbon (C-3) at δ 136.73.

On the basis of these spectral data compound 3 was identified as rhamnocitrin (kaempferol-7-O-methyl ether). It was further confirmed by comparison of spectral data with reported values [10] and co-TLC and MMP with an authentic sample.

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