

NEW PHENOLIC ACIDS FROM THE GALLS OF *TAMARIX APHYLLA* (L.) KARST.Mustafa Akhlaq¹, Ali Mohammed^{2*}¹Drug Standardization Research Unit (CCRUM), Faculty of Science, Jamia Hamdard, Hamdard Nagar, New Delhi-110062, India²Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi-110062, India

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*Mohammed Ali, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi-110 062, India, Email: maliphyto@gmail.com**ABSTRACT**

Phytochemical investigation of the galls of *Tamarix aphylla* (L.) Karst., syn. *T. articulata* Vahl. (Tamaricaceae) led to the isolation of three new phenolic acids identified as 3, 5- dihydroxy- 4 - (4'-hydroxybenzoxy) benzoic acid (aphyllaioic acid), 3, 5- dihydroxy – 4 - (2', 6'-diethyl-4'-hydroxymethylene benzoxy) benzoic acid (tamarixioic acid) and 3, 3'-dimethoxy- 4, 4'- bis – methyleneoxy - trans, trans - cinammic acid (dimethyleneoxy diferulic acid) along with known compounds β - sitosterol, ferulic acid and β - sitosterol - 3 β - D - glucoside. The structures of all the phytoconstituents was established on the basis of spectral data analyses and chemical reactions.

KEY WORDS: *Tamarix aphylla*, Tamaricaceae, galls, phenolic acids**INTRODUCTION**

Tamarix aphylla (L.) Karst., syn. *T. articulata* Vahl. (Tamaricaceae), known as lal – jhav, is a moderate size, up to 18 m tall with an erect, tapering trunk found in northern India, Gujarat, Iran, Saudi Arabia and Mediterranean region¹. It is grown in gardens for its delicately coloured inflorescence². Its twigs are attached by a gall forming insect. The galls are astringent and their aqueous extract is used as gargles to cure throat infection². Ellagitannins, phenolic acids and sugars are reported from the galls^{3,4}. Flavonoid glycosides⁵, brevifolin, carboxylic acid⁶, myricadiol, isomyricadiol⁷ and aphyllin⁸ are isolated from the different parts of the plant. This paper describes isolation and characterization of new phenolic acids from the galls of *T. aphylla*.

MATERIALS AND METHODS**General experimental procedures**

Melting points were determined on Perfit melting point apparatus and were uncorrected. UV spectra were recorded on Beckman DU-60 spectrophotometer in methanol. FT-IR spectra were measured on Jascp FT-IR-5000 spectrophotometer using KBr pelletes. ¹H NMR spectra were screened on advanced dry 400 Bruker spectrosin 400 MHz instrument using CDCl₃ as solvent and tetramethyl silane (TMS) as an internal standard. ¹³C NMR spectra were determined on advanced dry 400 Bruker spectrosin 100 MHz with TMS as an internal standard. Mass spectra (MS) were scanned by effecting

electron impact (EI) ionisation at 70 eV on a JEOL- JMS – DX – 303 instrument.

Plant material

The stem galls of *T. articulata* were procured from the Lucknow market and identified by the Botanist Dr. Raisuddin Ahmad (Research Officer), Drug Standardization Research Unit (CCRUM), Faculty of Science, Jamia Hamdard, Hamdard Nagar, New Delhi.

Extraction and isolation of compounds

The dried plant material (2 kg) was coarsely powdered and extracted with petroleum ether in the Soxhlet apparatus followed by extraction with ethyl alcohol exhaustively for 68 hours. The extract was concentrated to get a reddish brown coloured viscous mass, 658 g (33%). The ethanol extract was dissolved in a minimum amount of methanol and adsorbed on silica gel to form slurry. The slurry was air-dried, subjected to silica gel column chromatography and the column was eluted with chloroform and methanol in order of increasing polarity to isolate the following compounds:

 β -Sitosterol (1)

Elution of the column with chloroform furnished colourless amorphous powder of **1**, recrystallized from chloroform-methanol (1:1), 38 mg (0.002% yield), mp 138-140°C, R_f: 0.43 (chloroform - petroleum ether 3:1), IR ν_{\max} (KBr): 3430, 1620 cm⁻¹, ¹H NMR: δ 5.36 (1H, d, J=4.64 Hz, H-6), 3.52 (1H, brs, w_{1/2} 18.5 Hz, H-3 α), 1.01 (3H, brs, Me-10), 0.93 (3H, d, J=6.50, Hz, Me-21),

.0.84 (3H, t, J=6.03, Hz, Me-29), 0.82 (3H, d, J=6.00, Hz, Me-26), 0.80 (3H, d, J=6.0 Hz, Me-27), 0.68 (3H, brs, Me-18). ; EIMS m/z (rel. int.): 414 $[M]^+$ ($C_{29}H_{50}O$), (54.4)

Ferulic acid (2)

Elution of the column with $CHCl_3$ - MeOH (49 : 1) afforded greenish yellow powder of **2**, recrystallized from $CHCl_3$ - MeOH (9 : 1), 210 mg (0.0105% yield), R_f : 0.79 ($CHCl_3$ - EtOAc, 5 : 0.4) ; mp: 161-163°C ; UV λ_{max} (MeOH): 340 nm (log ϵ 5.2) ; IR ν_{max} (KBr): 3350, 3160, 1701, 1645, 1552, 850 cm^{-1} ; 1H NMR: (DMSO- d_6): δ 7.42 (1H, d, J=2.5 Hz, H-2), 6.91 (1H, d, J=8.7Hz, H-5), 6.27 (1H, dd, J=2.5, 8.7 Hz, H-6), 5.89 (1H, d, J=15.8 Hz, H-7), 5.73 (1H, d, J=15.8 Hz, H-8), 3.79 (3H, brs, OMe). ; ^{13}C NMR (DMSO- d_6): 144.22 (C-1), 116.32 (C-2), 149.89 (C-3), 161.52 (C-4), 127.13 (C-5), 121.05 (C-6), 114.10 (C-7), 112.02 (C-8), 179.84 (C-9), 55.65 (OMe). ; +ve FABMS m/z (rel. int.): 194 $[M]^+$ ($C_{10}H_{10}O_4$) (100).

Aphyllaoic acid (3)

Elution of the column with $CHCl_3$ - MeOH (19 : 1) furnished pale yellow granular powder of **3**, recrystallized from acetone : methanol (1 : 1), 279 mg (0.014% yield) ; R_f : 0.64 ($CHCl_3$: MeOH, 8.8 : 1.2) ; mp: 260-261°C ; UV λ_{max} (MeOH): 285, 297 nm, (log ϵ 5.2, 5.3) ; IR ν_{max} (KBr): 3350, 3210, 2925, 1690, 1551, 935 cm^{-1} ; 1H NMR (DMSO- d_6): δ 13.89 (1H, brs, COOH), 8.26 (2H, d, J=2.3 Hz, H-2, H-, 7.15 (2H, m, H-2', H-6'), 6.90 (2H, m, H-3' H-5'). ; ^{13}C NMR (DMSO- d_6): 139.85 (C-1), 121.36 (C-2), 164.53 (C-3), 143.50 (C-4), 162.75 (C-5), 121.36 (C-6), 180.96 (C-7), 145.66 (C-1'), 118.05 (C-2'), 111.51 (C-3'), 155.47 (C-4'), 111.51 (C-5'), 118.05 (C-6'). ; +ve FABMS m/z (rel. int.): 262 $[M]^+$ ($C_{13}H_{10}O_6$) (100), 245 (10.2), 185 (63.1), 169 (2.1), 124 (48.3), 93 (15.2), 77 (61.3).

Tamarixioic acid (4)

Elution of the column with $CHCl_3$ - MeOH (19 : 1) yielded pale yellow amorphous powder of **4**, recrystallized from MeOH, 65 mg (0.0032%, yield). ; R_f : 0.39 ($CHCl_3$: MeOH, 9.4 : 0.6), mp: 240-241°C. ; UV λ_{max} (MeOH): 301 nm (log ϵ 5.2). ; IR ν_{max} (KBr): 3510, 3300, 3195, 2950, 2845, 1690, 1560, 1455, 1121, 928, 849 cm^{-1} ; 1H NMR (DMSO - d_6) : δ 8.15 (2H, d, J=1.5Hz H-2, H-6,), 6.85 (2H, d, J=2.8 Hz, H-3', H-5'), 4.03 (2H, brs, H₂-9'), 2.53 (4H, brs, H₂-7', H₂-10'), 1.03 (3H, t, J=6.3 Hz, Me-8'), 0.99 (3H, t, J=6.1 Hz, Me-11'). ; ^{13}C NMR (DMSO- d_6): 138.31 (C-1), 119.59 (C-2), 165.82 (C-3), 145.52 (C-4), 163.80 (C-5), 119.59 (C-6), 181.75 (C-7), 145.52 (C-1'), 129.43 (C-2'), 108.47 (C-3'), 142.56 (C-4'), 108.47 (C-5'), 121.43 (C-6'), 38.94 (C-7'), 14.21 (C-8'), 64.09 (C-9'), 38.94 (C-10'), 14.24 (C-11'). ; EIMS m/z (rel. int.): 332 $[M]^+$ ($C_{18}H_{20}O_6$)

(29.9), 316 (19.9), 302 (15.3), 301 (14.7), 288 (7.1), 199 (31.3), 187 (41.4), 178 (14.5), 170 (28.3), 162 (7.2), 154 (82.9), 148 (10.1), 137 (11.8), 125 (18.3), 107 (13.6), 91 (10.1), 77 (14.3), 44 (100).

β -Sitosterol- β -D-glucoside (5)

Elution of the column with chloroform-methanol (9:1) furnished colourless amorphous powder of **5**, recrystallized from methanol. 104 mg (0.0052% yield), mp. 315-317°C, R_f 0.75 (chloroform – EtOAc, 9.2 : 0.8) ; IR ν_{max} (KBr): 3440, 3300, 3250, 1620, cm^{-1} . ; EIMS m/z (rel. int.) 576 $[M]^+$ ($C_{35}H_{60}O_6$) (4.2).

Dimethyleneoxydiferulic acid (6)

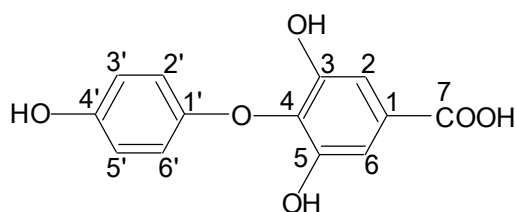
Elution of the column with $CHCl_3$ - MeOH (9:1) mixture gave light brown granular powder of **6**, recrystallized from $CHCl_3$: MeOH (1:1), 70 mg (0.0035% yield), R_f : 0.85 ($CHCl_3$), mp : 90-91°C; UV λ_{max} (MeOH): 212, 335 nm (log ϵ 4.15, 5.1), IR ν_{max} (KBr): 3170, 2950, 2845, 1690, 1635, 1580, 1465, 1095, 980 cm^{-1} . ; 1H NMR (DMSO- d_6): δ 13.25 (2H, brs, 2 x COOH), 8.27 (2H, d, J=2.8 Hz, H-2, H-2'), 7.70 (2H, d, J=8.5 Hz, H-5, H-5'), 7.23 (1 H, m, H-6), 7.16 (1H, m, H-6'), 6.16 (2H, d, J=15.8 Hz, H-7, H-7'), 6.05 (2H, d, J=15.8 Hz, H-8, H-8'), 4.05 (4H, brs, 2 x CH_2O), 3.81 (6H, brs, 2xMe) ; ^{13}C NMR (DMSO- d_6): δ 150.86 (C-1), 141.08 (C-2), 156.35 (C-3), 164.51 (C-4), 138.19 (C-5), 129.05 (C-6), 111.98 (C-7), 111.01 (C-8), 183.15 (C-9), 152.16 (C-1'), 140.19 (C-2'), 156.37 (C-3'), 164.50 (C-4'), 138.17 (C-5'), 128.95 (C-6'), 111.95 (C-7'), 111.08 (C-8'), 182.29 (C-9'), 60.85 (OMe), 60.83 (OMe), 65.45 (OCH_2CH_2O). ; +ve FABMS m/z (rel.int.): 414 $[M]^+$ ($C_{22}H_{22}O_8$) (68.3), 193 (100), 178 (48.3), 133 (35.8), 107 (24.1), 95 (21.0), 77 (18.2).

RESULT AND DISCUSSION

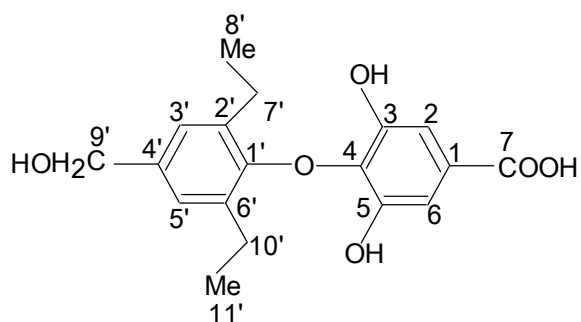
Compound 1, 2 and 5 are the known compounds identified as β - sitostero¹⁹, ferulic acid¹⁰ and β - sitosterol- 3 β -D - glucoside¹¹.

Compound 3, designated as aphyllaoic acid, was obtained as a pale yellow granular powder from chloroform - MeOH (19:1) eluants. It gave green colour with ferric chloride and produced effervescences with sodium bicarbonate solution. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3350 cm^{-1}), carboxylic function (3210, 1690 cm^{-1}) and aromaticity (1551, 935 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra, the molecular weight of 3 was determined as at m/z 262 corresponding to the molecular formula of a hydroxyphenyl substituted gallic acid, $C_{13}H_{10}O_6$. The ion peaks arising at m/z 245 $[M-OH]^+$, 169 $[M-C_6H_4OH]^+$ and 93 $[C_6H_4OH]^+$ suggested the linkage of the hydroxybenzyl ring to gallic acid. The 1H NMR spectrum of 3 showed a two-proton doublet at 8.26 (J=2.3 Hz) assigned to meta - coupled aromatic H-2

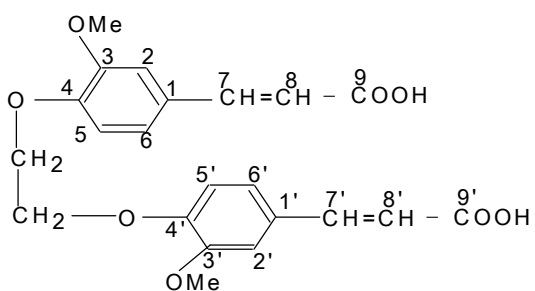
and H-6 protons. Two multiplets at β 7.15 and 6.90 integrating for two protons each were ascribed to aromatic H-2', H-6' and H-3', H-5' protons. The ^{13}C NMR spectrum of **3** exhibited signals for carboxylic carbon at β 180.96 (C-7) and aromatic carbons from β 164.53 to 111.51. The HMBC spectrum of **3** showed correlations C-1 with H-2 / H-6 ; C-3 / C-5 with H-2 / H-6 ; C-1' with H-2' / H-6' and C-4' with H-3' / H-5'. On the basis of above discussion, the structure of **3** has been elucidated as 3, 5--dihydroxy-4 - (4'-hydroxybenzoxy) benzoic acid. This is a new gallic acid derivative.



3



4



6

Compound **4**, named tamarixic acid, was obtained as pale yellow amorphous powder from chloroform - methanol (19:1) eluents. It gave green colour with ferric chloride due to phenolic nature and produced effervescences with sodium bicarbonate solution. Its IR spectrum showed characteristic absorption bands for hydroxyl groups ($3510, 3300\text{ cm}^{-1}$) and carboxylic group

($3195, 1690\text{ cm}^{-1}$). On the basis of FAB mass and ^{13}C NMR spectra, the molecular weight of **4** was determined as at m/z 332 consistent with a diethyl benzyl alcohol substituted gallic acid $\text{C}_{18}\text{H}_{20}\text{O}_6$. The ion peaks arising at m/z 316 $[\text{M} - \text{Me}]^+$, 302 $[\text{M} - \text{C}_2\text{H}_5]^+$, 301 $[\text{M} - \text{CH}_2\text{OH}]^+$, 170 $[\text{C}(\text{OH})_2\text{C}_6\text{H}_2 - \text{COOH}]^+$, 162 $[\text{M} - 170]^+$, 154 $[\text{OH}]_2 - \text{C}_6\text{H}_2\text{COOH}]^+$ and 178 $[\text{M} - 154]^+$, indicated that diethyl benzyl alcohol was linked to gallic acid. The ^1H NMR spectrum of **4** exhibited two doublet at δ 8.15 ($J=1.5\text{ Hz}$) and 6.85 ($J=2.8\text{ Hz}$), integrated for two protons each, assigned to meta-coupled H-2, H-6 and H-3', H-5' protons, respectively. A two-proton broad signal at δ 4.03 and a four-proton broad signal at δ 2.53 were attributed to hydroxymethylene H_2-9' and methylene H_2-7' and H_2-10' protons, respectively. Two three-proton triplets δ 1.03 ($J=6.3\text{ Hz}$) and 0.99 ($J=6.1\text{ Hz}$) were accounted to C-8' and C-11' primary methyl protons, respectively. The ^{13}C NMR spectrum of **4** exhibited signals for carboxylic carbon at δ 181.75 (C-7), aromatic carbons from δ 165.82 to 108.47, hydroxymethylene carbons at δ 64.09 (C-9'), methylene carbons at δ 34.94 and methyl carbons at 14.21 (C-8') and 14.24 (C-11'). The HMBC spectrum of **4** showed correlations of C-7 with H-2 / H-6 ; C-2' / C-6' with $\text{H}_2-7' / \text{H}_2-10'$ and H-3' / H-5'; and C-4' with H-3' / H-5' and H_2-9' . The $^1\text{H} - ^1\text{H}$ COSY spectrum of **4** exhibited interactions of $\text{H}_2-7' / \text{H}_2-10'$ with $\text{H}_3-8' / \text{H}_3-11'$ and H-3' / H-5'; and H_2-4' with H-3' / H-5'. On the basis of spectral data analysis, the structure of **4** has been elucidated as 3, 5 - dihydroxy - 4 - (2', 6'- diethyl - 4'-hydroxymethylene benzoxy) benzoic acid. It is a new gallic acid derivative.

Compound **6**, named dimethyleneoxydiferulic acid, was obtained as a light brown granular powder from chloroform - MeOH (9:1) eluents. It decolourised bromine water due to unsaturation and produced effervescences with sodium bicarbonate solution. Its IR spectrum showed characteristics absorption bands for carboxylic groups ($3170, 1690\text{ cm}^{-1}$), unsaturation (1635 cm^{-1}) and aromatic nucleus ($1580, 980\text{ cm}^{-1}$). The FAB mass displayed a molecular ion peak at m/z 414 consistent to a molecular formula of diethylene diferulic acid, $\text{C}_{22}\text{H}_{22}\text{O}_8$. The ion peaks arising at m/z 193 $[\text{O}(\text{OMe})\text{C}_6\text{H}_3\text{CH}=\text{CHCOOH}]^+$, 178 $[\text{M} - 178]^+$, 133 $[\text{M} - 178 - \text{COOH}]^+$ and 107 $[\text{M} - 133 - \text{CH}=\text{CH}]^+$ supported the presence of ferulic acid linkage in the molecule. The ^1H NMR spectrum of **6** exhibited two doublets at δ 8.27 ($J=2.8\text{ Hz}$) and 7.70 ($J=8.5\text{ Hz}$), integrating for two - protons each, assigned to meta-coupled H-2, H-2' and to ortho-coupled H-5, H-5' protons, respectively. Two one-proton multiplets at δ 7.23 and 7.16 were ascribed to aromatic H-6 and H-6', respectively. Two doublets at δ

6.16 and 6.05 with coupling interactions of 15.8 Hz each were accounted to trans - oriented H-7, H-7' and H-8, H-8' protons, respectively. A four-proton broad signal at δ 4.05 and a six-proton broad signal at δ 3.81 were attributed correspondingly to oxygenated methylene and methoxy protons. The ^{13}C NMR spectrum of **6** showed signals for aromatic carbons from δ 164.51 to 128.95, vinylic carbons from δ 111.98 to 111.01, carboxylic carbons at δ 183.15 and 182.29, methoxy carbons at δ 60.85 and 60.83 and oxygenated methylene carbons at δ 65.45. The HMBC spectrum of **6** showed correlations of C-3 / C-3' with H-2 / H-2' and OMe; C-4 / C-4' with OCH₂ and H-5 / H-5'; C-9 / C-9' with H-8 / H-8'; and C-1 / C-1' with H-2 / H-2', H-6 / H-6' and H-7 / H-7'. On the basis of the foregoing account the structure of **6** was determined as 3, 3'- dimethoxy-4, 4'-bis - methyleneoxy - trans, trans - cinammic acid. This is a new cinammic acid derivative.

CONCLUSION

The findings of the present work have shown that the stem galls of *T. aphylla* are rich in phenolic compounds which may show antioxidant activity.

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