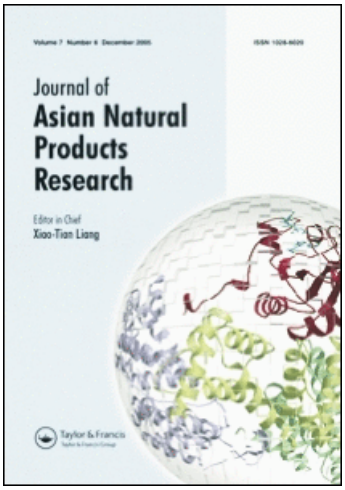

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Chemical constituents from the seeds of *Zanthoxylum alatum*

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Chemical studies on the seeds of *Zanthoxylum alatum* Roxb. (Rutaceae) led to the isolation of two new phenolic constituents characterized as 3-methoxy-11-hydroxy-6,8-dimethylcarboxylate biphenyl (**3**) and 3,5,6,7-tetrahydroxy-3′,4′-dimethoxyflavone-5-β-D-xylopyranoside (**6**), along with the five known compounds, 1-methoxy-1,6,3-anthraquinone, 1-hydroxy-6,13-anthraquinone, 2-hydroxybenzoic acid, 2-hydroxy-4-methoxy benzoic acid, and stigmasta-5-en-3β-D-glucopyranoside, on the basis of spectral data and chemical analyses.

Keywords: *Zanthoxylum alatum* Roxb.; Rutaceae; diphenyl alatumoic acid diester; zanthoxylumflavone xyloside; phenolic constituents

1. Introduction

Zanthoxylum alatum Roxb. (Rutaceae) is an erect shrub or a small tree commonly occurring in hot valleys of the Himalayas. It is extensively used in the Indian system of medicine as stomachic, carminative, and anthelmintic agents. The fruits and seeds are employed as an aromatic tonic in fever, dyspepsia, and cholera. The seeds possess deodorant, disinfectant, and antiseptic properties and are used to relieve toothache and their lotions for scabies [1].

A volatile oil consisting mainly linalool [2], monoterpenetriol-3,7-dimethyl-1-octane-3,6,7-triol, *trans*-cinnamic acid, nevadensin, umbelliferone, β-sitosterol and its glycoside [3], 3,5-dihydroxy-7,8,4′-trimethoxyflavone (tambulin) and tambuletin [4], and unsaturated fatty acids [5] has been reported from the seeds. Biological activities, *viz.* antibacterial [6], antifungal [7], anthelmintic [8], antidiabetic [9], antiproliferative [10], and insecticidal [11], have been studied. In the present paper, we report the isolation and characterization of two new phenolic constituents and five known compounds from the seeds of *Z. alatum*.

2. Results and discussion

Compounds **1**, **2**, **4**, **5**, and **7** were the known phytoconstituents identified as 1-methoxy-6,13-anthraquinone, 1-hydroxy-6,13-anthraquinone, 2-hydroxybenzoic acid, 2-hydroxy-4-methoxy-benzoic acid, and stigmasta-5-en-3β-D-glucopyranoside, respectively.

Compound **3**, named diphenyl alatumoic acid dimethyl ester, was obtained as pale yellow crystals from chloroform eluants. It gave blue color with alcoholic ferric chloride solution indicating the phenolic nature of the molecule. Its IR spectrum exhibited distinctive absorption bands for hydroxyl group (3309 cm^{-1}), ester group (1721 cm^{-1}), and aromatic rings (1640, 1601, 1564, and 830 cm^{-1}). It had a molecular ion peak at *m/z* 316 in its mass spectrum corresponding to a molecular formula of a disubstituted biphenyl ester-type molecule, $C_{17}H_{16}O_6$

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confirmed by HR-MS at *m/z* 317.2896 $[M+H]^+$. The ^1H NMR spectrum of **3** displayed two one-proton *ortho*-coupled doublets at δ 8.19 ($J = 8.7$ Hz) and 8.16 ($J = 8.7$ Hz) assigned to H-5 and H-9, respectively; two one-proton *meta*-coupled doublets at δ 6.53 ($J = 2.1$ Hz) and 6.37 ($J = 2.3$ Hz) ascribed to H-12 and H-2, respectively; and two one-proton *ortho*-, *meta*-coupled double doublets at δ 6.97 ($J = 8.7$, 2.1 Hz) and 6.90 ($J = 8.7$, 2.3 Hz) attributed correspondingly to H-10 and H-4, respectively. Two broad signals of three-proton each at δ 3.88 and 3.85 were attributed to methyl ester proton and a broad signal of three-proton resonating at δ 3.80 was associated with methoxy proton. Two deshielded carbon signals in the ^{13}C NMR spectrum of **3** at δ 175.5 and 173.5 and upfielded carbon signals at δ 56.4 and 55.4 were attributed to methyl ester carbon (C-6 and C-8). The carbon signals appearing between δ 161.2 and 95.1 were ascribed to aromatic carbons and the signal at δ 61.6 was accounted for C-3 methoxy carbon. The $^1H-^1H$ COSY spectrum of **3** showed correlation of H-4 with H-5 and H-9 with H-10. The HMBC spectrum of **3** exhibited correlation of C-6 with H-5 and H-4; C-3 with H-2 and H-4; C-8 with H-9 and H-10; (a) CO with H-5; (b) CO with H-9; and C-11 with H-12. On the basis of the spectral data analyses, the structure of **3** has been elucidated as 3-methoxy-11-hydroxy-6,8-dimethylcarboxylate biphenyl (Figure 1).

Compound **6**, named zanthoxylumflavone xyloside, was obtained as pale yellow amorphous powder from chloroform–methanol (9:1) eluants. It gave positive test for flavonoids. Its UV absorption maxima at 258 and 381 nm were typical of 3-hydroxy substituted flavones [12]. It showed shift of bands with sodium methoxide suggesting the presence of free hydroxyl groups in the molecule. A shift of band II with sodium acetate solution indicated the location of free hydroxyl group at C-7. Absence of any shift of band with aluminum chloride supported the existence of 4′-methoxy group. Absence of

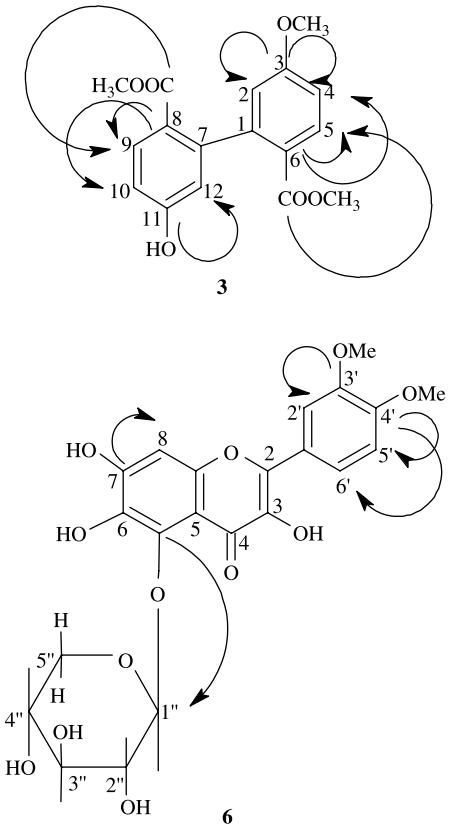

Figure 1. Structures of compounds **3** and **6**.

any significant shift in band I with sodium acetate and boric acid ruled out the existence of B-ring *O*-dihydroxy groups. Its IR spectrum exhibited characteristic absorption bands for hydroxyl groups (3432, 3314, and 3127 cm^{-1}) and carbonyl group (1670 cm^{-1}). Its FAB mass spectrum exhibited a molecular ion peak at *m/z* 478 consistent with the molecular formula of flavonol glycoside, $C_{22}H_{22}O_{12}$, confirmed by HR-MS at *m/z* 479.4119 $[M+H]^+$. The generation of important ion peak at *m/z* 300 [M-178, O—C_2, C_3—C_4 fission]$^+$ indicated the presence of the hydroxyl group at C-3 and the attachment of two methoxyl groups with aromatic ring B. The ion fragments appearing at *m/z* 167 [M-178 − $C_5H_9O_4]^+$, 151 [M-178 − $C_5H_9O_5]^+$, and 134 [151-OH]$^+$ suggested the attachment of xylosyl group to the aromatic ring A. The other prominent ion peaks arose at *m/z* 463

$[M-Me]^+$, 447 $[M-OMe]^+$, 416 [447-OMe]$^+$, and 149 $[C_5H_9O_4]^+$ supporting the existence of two methoxyl and one xylosyl groups in the molecule. The ^{1}H NMR spectrum of **6** displayed two one-proton doublets at δ 7.96 ($J = 2.3$ Hz) and 7.15 ($J = 7.4$ Hz) assigned to *meta*-coupled H-2′ and *ortho*-coupled H-5′, respectively. A one-proton double doublet at δ 6.69 ($J = 2.3$, 7.4 Hz) was accounted to *ortho*-, *meta*-coupled H-6′, suggesting ABX spin system of ring B. A one-proton broad signal at δ 6.65 was ascribed to aromatic proton H-8. The two methoxyl groups resonated as a broad signal of six protons at δ 3.45. A one-proton doublet at δ 5.34 ($J = 7.1$ Hz) was attributed to the anomeric proton H-1″. The hydroxymethine protons of the sugar moiety appeared between δ 5.17 and 4.85. The oxygenated methylene protons resonated as two doublets of one proton each at δ 3.97 ($J = 11.4$ Hz) and 3.93 ($J = 11.4$ Hz) ascribed to H-5″a and H-5″b. The ^{13}C NMR spectrum of **6** showed important signal for carbonyl carbon at δ 176.3 (C-4) supporting the flavonol-type carbon framework of the molecule. The carbon signals at δ 56.7 and 55.6 supported the existence of two methoxyl groups in the molecule. Oxygenated aromatic carbons appeared at δ 145.9 (C-3′), 149.4 (C-4′), 157.7 (C-5), 154.1 (C-6), 163.9 (C-7), 156.2 (C-9), and 136.0 (C-3). The sugar carbons resonated between δ 100.3 and 61.3. The ^{1}H and ^{13}C NMR data of **6** were compared with the flavonol molecules [13–15]. The ^{1}H–^{1}H COSY spectrum of **6** showed correlation of H-5′ with H-6′; H-2′ with H-6′; H-1″ with H-2″ and H-3″; and H_2-5″ with H-4″ and H-3″. In HMBC spectrum, C-7 correlated with H-8; C-3′ correlated with H-2′; C-4′ correlated with H-5′ and H-6′; and C-5 correlated with H-1″. Acid hydrolysis of flavonol glycoside yielded flavonol and sugar as xylose (TLC comparable). On the basis of the spectral data analysis and chemical reactions, the structure of **6** has been established as 3,5,6,7-tetrahydroxy-3′,4′-dimethoxyflavone-5-β-D-xylopyranoside (Figure 1).

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Perfit melting point apparatus (Ambala, Haryana, India) and are uncorrected. The IR spectra were recorded on KBr discs, using a Bio-Rad FT-IR 5000 spectrometer (FTS 135, Kowloon, Hong Kong). The UV spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin-Elmer, Rotkreuz, Switzerland) in methanol. The ^{1}H and ^{13}C NMR spectra were scanned using Bruker Advance DRY 400 spectrospin and Bruker Advance DRY 100 spectrospin instruments (Karlesrühe, Germany), respectively, in $CDCl_3$ and TMS as an internal standard. FAB-MS spectra were obtained using JEOL-JMS-DX 303 spectrometer (Japan). Column chromatography was performed on silica gel (60–120 mesh; Qualigens, Mumbai, India) . TLC was run on silica gel G (Qualigens). Spots were visualized by an exposure to iodine vapors, UV radiation, and spraying reagents.

3.2 Plant material

The seeds of *Z. alatum* were procured from the Khari Baoli local market of Delhi and identified by Dr M.P. Sharma, Taxonomist, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen No. PRL/JH/04/05 is deposited in the Herbarium of the Phytochemical Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi.

3.3 Extraction and isolation

The seeds (2 kg) dried at 45°C in an electric oven were coarsely powdered and exhaustively extracted with petroleum ether (60–80°C) in a Soxhlet apparatus to remove all the fatty components (200 g, 10.0%). The defatted material was re-extracted with ethanol. The extract was concentrated under rotatory evaporator to yield a dark brown viscous mass (200 g, 10.0%).

The extract was dissolved in 250 ml methanol and adsorbed on silica gel (60–120 mesh) for preparation of slurry.

The air-dried slurry was chromatographed over the silica gel column packed in petroleum ether (60–80°C). The column was eluted with petroleum ether, petroleum ether–chloroform (9:1, 3:1, 1:1, 1:3, v/v), chloroform, chloroform–methanol (99:1, 98:2, 95:5, 9:1, 3:1, 1:1, 1:3, v/v) and methanol, successively, in the order of increasing polarity to isolate the following compounds.

3.3.1 *Methoxyanthraquinone* (**1**)

Elution of the column with chloroform afforded pale yellow crystals of **1**, recrystallized from acetone; 166 mg (0.0083%); R_f: 0.53 (chloroform); mp 116–118°C; UV λ_{max} (MeOH): 273 nm (log ε 6.1); IR (ν_{max} (KBr): 2919, 2855, 1696, 1630, 1604, 1577, 1423, 1311, 1286, 1263, 1221, 1169, 980, 943, and 769 cm^{-1}; positive FAB-MS *m/z* (rel. int.): 238 $[M]^+$ ($C_{15}H_{10}O_3$) (3.2).

3.3.2 *Hydroxyanthraquinone* (**2**)

Elution of the column with chloroform yielded light brown crystals of **2**, recrystallized from methanol; 128 mg (0.0064%); R_f: 0.45 (chloroform); mp 127–128°C; UV λ_{max} (MeOH): 280 nm (log ε 6.1); IR ν_{max} (KBr): 3380, 2920, 2850, 1702, 1637, 1560, 1449, 1419, 1314, 1284, 1221, 979, and 769 cm^{-1}; positive FAB-MS *m/z* (rel. int.): 224 $[M]^+$ ($C_{14}H_8O_3$) (9.2).

3.3.3 *Diphenyl alatumoic dimethyl ester* (**3**)

Further elution of the column with chloroform furnished pale yellow crystals of **3**, recrystallized from methanol, 422 mg (0.0211%); R_f: 0.55 (chloroform); mp 209–210°C; UV λ_{max} (MeOH): 230 and 275 nm (log ε 4.9 and 2.8); IR ν_{max} (KBr): 3309, 2932, 1721, 1660, 1601, 1564, 1510, 1314, 1260, 1190, 1133, 1032, and 830 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.19 (1H, d, $J = 8.7$ Hz, H-5), 8.16 (1H, d, $J = 8.7$ Hz, H-9), 6.97 (1H, dd, $J = 8.7$, 2.1 Hz, H-10), 6.90 (1H, dd, $J = 8.7$, 2.3 Hz, H-4), 6.53 (1H, d, $J = 2.1$ Hz, H-12), 6.37 (1H, d, $J = 2.3$ Hz, H-2), 3.88 (3H, brs, $COOCH_3$), 3.85 (3H, brs, $COOCH_3$), and 3.80 (3H, brs, OCH_3); ^{13}C NMR ($CDCl_3$): δ 175.7 ($COOCH_3$), 173.5 ($COOCH_3$), 161.2 (C-3), 158.5 (C-11), 135.4 (C-6, C-8), 129.5 (C-4), 123.3 (C-10), 114.2 (C-1, C-7), 104.4 (C-2), 98.4 (C-12), 95.1 (C-5, C-9), 61.6 (OCH_3), 56.4 ($COOCH_3$), and 55.4 ($COOCH_3$); HR-FAB-MS *m/z* 317.2896 $[M+H]^+$ (calcd for $C_{17}H_{16}O_6$, 317.2987).

3.3.4 *Salicylic acid* (**4**)

Elution of the column with chloroform–methanol (49:1) yielded colorless crystals of **4**, recrystallized from chloroform–methanol (1:1); 77 mg (0.0038%); R_f: 0.58 (toluene–ethyl acetate–formic acid; 5:4:1); mp 157–159°C. (lit. mp 157–159°C) (Merck Index, 2001); UV λ_{max} (MeOH): 230 nm (log ε 4.3); IR ν_{max} (KBr): 3450, 2919, 2850, 1682, 1622, 1567, 1411, 1323, 1236, 1136, and 834 cm^{-1}; positive FAB-MS *m/z* (rel. int.): 138 $[M]^+$ ($C_7H_6O_3$) (62.8).

3.3.5 *Methoxysalicylic acid* (**5**)

Further elution of the column with chloroform–methanol (49:1) furnished light brown crystals of **5**, recrystallized from acetone; 95 mg (0.0047%); R_f: 0.51 (toluene–ethyl acetate–formic acid; 5:4:1); mp 238–240°C; UV λ_{max} (MeOH): 224 nm (log ε 5.1); IR ν_{max} (KBr): 3421, 2919, 2852, 1690, 1640, 1516, 1448, 1422, 1356, 1309, 1221, 1129, 1090, 1018, 926, and 762 cm^{-1}; positive FAB-MS *m/z* (rel. int.): 168 $[M]^+$ ($C_8H_8O_4$) (27.8), 153 (19.6), 151 (17.2), 137 (42.8), 136 (71.6), 123 (12.3), 120 (9.7), 108 (11.2), and 107 (27.9).

3.3.6 *Zanthoxylumflavone xyloside* (**6**)

Elution of the column with chloroform–methanol (9:1) afforded pale yellow amorphous powder of **6**, recrystallized from methanol, 150 mg (0.0075%); R_f: 0.38 (toluene–ethyl acetate–formic acid; 5:4:1); mp 263–264°C; UV λ_{max} (MeOH): 258 and 381 nm (log ε 4.9 and 1.8); λ_{max} (MeOH + NaOMe):

273 and 437 nm; λ_{max} (MeOH + NaOAc): 268 and 437 nm. λ_{max} (MeOH + NaOAc + H_3BO_3): 261 and 386 nm; λ_{max} (MeOH + $AlCl_3$): 241 and 362 nm; λ_{max} (MeOH + $AlCl_3$ + HCl): 231 and 354 nm; IR ν_{max} (KBr): 3432, 3314, 3127, 2920, 2841, 1670, 1604, 1550, 1514, 1435, 1344, 1265, 1214, 1170, 1124, 1075, 1032, and 981 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.96 (1H, d, $J = 2.3$ Hz, H-2′), 7.15 (1H, d, $J = 7.4$ Hz, H-5′), 6.69 (1H, dd, $J = 2.3, 7.4$ Hz, H-6′), 6.65 (1H, brs, H-8), 5.34 (1H, d, $J = 7.1$ Hz, H-1″), 5.17 (1H, m, H-2″), 5.04 (1H, m, H-3″), 4.85 (1H, m, H-4″), 3.97 (1H, d, $J = 11.4$ Hz, H-5″a), 3.93 (1H, d, $J = 11.4$ Hz, H-5″b), and 3.45 (6H, brs, 2× OMe); ^{13}C NMR (DMSO-d_6): δ 148.3 (C-2), 136.0 (C-3), 176.3 (C-4), 157.7 (C-5), 154.1 (C-6), 163.9 (C-7), 95.3 (C-8), 156.2 (C-9), 103.3 (C-10), 120.2 (C-1′), 123.7 (C-2′), 145.9 (C-3′), 149.4 (C-4′), 115.5 (C-5′), 111.6 (C-6′), 100.3 (C-1″), 76.3 (C-2″), 74.3 (C-3″), 70.2 (C-4″), 66.0 (C-5″), 56.7 (OMe), and 55.6 (OMe); HR-FAB-MS 479.4119 $[M + H]^+$ (calcd for $C_{22}H_{22}O_{12}$ 479.4138).

3.3.6.1 Acid hydrolysis of **6**. Compound **6** (20 mg) was dissolved in methanol and 2 N HCl (1:1) and heated this solution up to half volume left. The solution was extracted with ethyl acetate (3× 10 ml), washed with water (2× 10 ml), dried it over Na_2SO_4, and evaporated to obtain the aglycone. The aqueous phase was concentrated and subjected to paper chromatography using butanol:ethanol:water (4:1:2.2) as the developing solvent system for standard samples of monosaccharide. The paper was sprayed with aniline hydrogen phthalate and the sugar was identified as xylose.

3.3.7 β-Sitosterol-β-D-glucoside (**7**)

Elution of the column with chloroform–methanol (19:1) produced colorless amorphous powder of **7**, recrystallized from methanol, 103 mg (0.005%); R_f: 0.76 (chloroform–ethanol; 9.5:0.5); mp 315–320°C; UV λ_{max} (MeOH): 207 nm (log ε 4.15); IR ν_{max} (KBr): 3440, 3300, 3250, 2923, 2852, 1620, 1464, 1377, 1121, 1055, 955, 858, and 785 cm^{-1}. Positive FAB-MS *m/z* (rel. int.) 576 $[M^+]$ ($C_{35}H_{60}O_6$) (5.1).

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References

[1] Anonymous, *The Wealth of India, A Dictionary of Indian Raw Materials and Industrial Products* (CSIR/PID, New Delhi, 2003), Vol. XI, pp. 17–21.
[2] R. Ramidi and M. Ali, *J. Essent. Oil Res.* **3**, 467 (1998).
[3] S.K. Talapatra, C.D. Sambhu, and B. Talapatra, *Indian J. Chem.* **28B**, 356 (1989).
[4] A.G.R. Nair, G.A. Nair, and C.P. Joshua, *Phytochemistry* **21**, 483 (1982).
[5] A. Ahmad, L.N. Misra, and M.M. Gupta, *J. Nat. Prod.* **56**, 456 (1993).
[6] N. Jain, S.K. Srivastava, K.K. Aggarwal, S. Ramesh, and S. Kumar, *Flav. Fragr. J.* **16**, 408 (2001).
[7] A. Dikshit and A. Husain, *Fitoterapia* **55**, 171 (1984).
[8] M.B. Mehta, M.D. Kharya, R. Srivastava, and K.C. Verma, *Indian Perf.* **25**, 19 (1981).
[9] M.M. Alam, M.B. Siddiqui, and W. Husain, *Fitoterapia* **61**, 240 (1990).
[10] S. Kumar and K. Miller, *Phytother. Res.* **13**, 214 (1999).
[11] M. Tiwary, S.N. Naik, D.K. Tiwary, P.K. Mittal, and S. Yadav, *J. Vect. Borne Dis.* **44**, 198 (2007).
[12] K.R. Markham, *Techniques of Flavonoids Identification* (Academic Press, London, 1982), pp. 36–45.
[13] W. Schliemann, B. Schneider, V. Wray, J. Schmidt, M. Nimtz, A. Porzel, and H. Bohm, *Phytochemistry* **67**, 191 (2006).
[14] M. Saleem, H.J. Kim, C.K. Han, C. Jin, and Y.S. Lee, *Phytochemistry* **67**, 1390 (2006).
[15] C. Mariani, A. Braca, S. Vitalini, N.D. Tommasi, F. Visioli, and G. Fico, *Phytochemistry* **69**, 1220 (2008).