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CONSTITUENTS FROM *Feronia Limonia*

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abstract: The root and stem bark of *Feronia limonia* yielded 5, 7-dihydroxy-3', 4'-dimethoxy -6, 8-di (3-methylbut-2-enyl) flavanone along with several known compounds viz., stigmasterol, friedelin, β-sitosterol-3-*O*-β-*D*-glucopyranoside, bergapten, xanthotoxin, scopoletin, isoimperatorin, osthol and 6, 7-dimethoxycoumarin. These compounds were characterized on the basis of U.V, IR, N.M.R (^{1}H, ^{13}C) and mass spectral studies.

key words: *Feronia limonia*; Rutaceae; flavanone; sterol; coumarin.

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1. Introduction

Feronia limonia Swingle belongs to the family Rutaceae (syns. *F. elephantum* Correa; *Limonia acidissima* L.: *Schinus limonia* L) commonly known as wood-apple, elephant apple, monkey fruit, curd fruit and *katha bel* in India [1-4].

The different parts of the plant have been investigated by several workers. Roots, leaves and stem bark contain coumarins and steroids, whereas ursolic acid and flavanone glycoside have been isolated from heartwood [5-9]. As a part of our study of some medicinal plants of the family Rutaceae found in Shahjahanpur district, phytochemical on *Feronia limonia* was carried out [10-12].

2. Experimental

General procedures

All melting points were measured on a Perfit melting point apparatus and uncorrected. Ultra violet absorption spectrum was recorded in methanol on Perkin-Elmer Lambda Bio 20 UV spectrometer. The IR spectrum was obtained on a Perkin-Elmer 1710 infrared Fourier transformation spectrometer using the KBr disc method. NMR spectra were recorded on

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Bruker AVANCE DRX- 300(300, 100 Hz). Chemical shifts are shown in δ values (ppm.) with tetramethylsilane (TMS) as an internal reference. FEBMS was recorded on JEOLSX 1021/DA-6000 mass spectrometer. Column chromatography was carried using silica gel (60-120 mesh). Chemicals are of analytical-reagent grade were purchased from E-Merck (India).

Plant material

The roots and stem bark of *Feronia limonia* were collected from the rural areas of the Shahjahanpur district in the month of November. Authentication was achieved by the comparison with the herbarium specimen deposited in the herbarium of the faculty of botany, G. F. College (Rohilkhand University), Shahjahanpur. Freshly harvested and dried material was used since old dried material stored for a period may undergo some qualitative changes. Healthy roots and stem bark were spread out and dried in the laboratory at room temperature until they broke easily by hand.

Extraction and Isolation

The air-dried plant material (roots and stem bark) of *Feronia limonia* (1.9 Kg) was extracted with methanol. Different extracts were combined and concentrated under reduced pressure. The residue (90 g) was suspended in methanol/water (1 L, 1:9, v/v) solution and extracted with petroleum ether, chloroform, acetone and ethyl acetate to give their extracts and aqueous phases. Out of these extracts chloroform, acetone and ethyl acetate extracts were considered for further investigation. These extracts were separately column chromatographed using silica gel and were eluted with different solvent system of increasing polarity. Several fractions were obtained in each of chromatography. These fractions were monitored with TLC and the fractions of similar TLC results were combined together. These combined fractions on rechromatography afforded several compounds. From chloroform three, from acetone six and from ethyl acetate only one compound could be isolated in pure form.

From chloroform extract by eluting the column with *n*-hexane/ethyl acetate (9:1) stigmasterol (FL-**1, 7 mg**), $CHCl_3$/MeOH (9:4) friedelin (FL-**2, 13 mg**) and from the eluent $CHCl_3$/MeOH (9:7) β-sitosterol-3-*O*- β -*D*-glucopyranoside (FL-**3, 15 mg**) was isolated. The UV, IR, MS and NMR data of these compounds (FL-1 to FL-3) were in good concurrence with those reported in literature [13-15].

From acetone extract by eluting the column with petrol-ether/ ethyl acetate (8:2) bergapten (FL-**4, 27 mg**), petrol-ether/ ethyl acetate (6:4) xanthotoxin **(**FL-**5, 35.4 mg**), chloroform/ ethyl acetate (8:2) scopoletin (FL-**6, 11.2** mg), petrol-ether/ ethyl acetate (8:2) isoimperatorin (FL-**7, 12 mg),** petrol-ether/ ethyl acetate (8:2) osthol (FL-**8, 5.6 mg**), and petrol-ether/ ethyl acetate (8:2) 6, 7-dimethoxycoumarin **(**FL-**9, 2 mg).**

Coumarins could be readily identified by direct comparison of their UV, IR, MS and NMR data with those published for the bergapten xanthotoxin scopoletin isoimperatorin osthol and 6, 7-dimethoxycoumarin respectively [16-18]. Similarly ethyl acetate extract on elution with acetone/ ethyl acetate (7:5) yielded 5, 7-dihydroxy-3', 4'-dimethoxy -6, 8-di (3-methylbut-2-enyl) flavanone **(**FL-10, **15.4 mg**).

Stigmasterol (FL-**1)**: white needles; mp. 169-170 ºC; IR λ_{max} (KBr) cm^{-1}: 3400, (OH), 3026, 1410, 1245, 815, 690; ^{1}H NMR ($CDCl_3$) δ: 5.33 (1H, H-6), 5.15 (2H, m ,H-22, H-23), 3.27 (1H, s, H-23), 0.90 (3H,*d*, *J* = 6.5 Hz, H-21), 0.87 (3H,*d*, *J* = 6.6 Hz, H-26), 0.84 (3H, *d*, *J* = 7.0 Hz, H-29), 0.81 (3H, *d*, *J* = 6.5 Hz, H-27), 0.80 (3H,s, H-19), 0.65 (3H,s,H-18); MS *m/z* (%): 412 $[M]^+$ (15), 397 $[M –Me]^+$ (10), 394 $[M –H_2O]^+$ (32), 379$[M –H_2O–Me]^+$ (79), 369 $[M–C_3H_7]^+$ (14), 351$[M –H_2O–C_3H_7]^+$ (60), 327 $[M–H_2O –C_5H_7]^+$(67), 300 $[M–C_8H_{16}]^+$(54),

Friedelin(FL-**2):** white needles; mp. 261-262 ºC; IR λ_{max} (KBr) cm^{-1}: 3421, (OH), 2938, 2864, 2360, 1650 (C=C), 1465, 1382, 1241, 1192, 1133, 1107, 1062; ^{1}H NMR ($CDCl_3$) δ: 2.40 (ddd, *J* = 13.96, 5.26, 2.06 Hz, H-2), 2.31 (ddd, *J* = 13.96, 7.09, 1.15 Hz, H-2), 2.25 (d, *J* = 6.64 Hz, H-4), 1.18 (3H, s, H-28), 1.05 (3H, s, H-27), 1.01 (3H, s, H-26), 1.00 (3H, s, H-30), 0.96 (3H, s, H-29), 0.88 (d, *J* = 6.64 Hz, H-23) and 0.87 (3H, s, H-25), 0.73 (3H, s, H-24)

β -sitosterol-3-*O*- β -*D*-glucopyranoside (FL-**3):** white crystal; mp 280-282 °C; IR ν_{max} (KBr) cm^{-1}: 3460, (OH), 3035, 1654 (C=C); ^{1}H NMR ($CDCl_3$) δ: 5.34 (1H,*d*, *J* = 2.1 Hz, H-1'), 5.14 (1H,*d*, *J* = 5.6 Hz, H-1'), 4.53(1H, s, H-6'), 4.27(1H, s, H-3'), 4.52(1H, s, H-4'), 4.03(1H, s, H-2'), 3.96(1H, s, H-5'), 3.85(1H, s, H-3), 1.02 (3H,s, H-19), 0.92 (3H,*d*, *J* = 6.4 Hz, H-21), 0.86 (3H,*d*, *J* = 7.3 Hz, H-29), 0.83 (3H,*d*, *J* = 6.8 Hz, H-26), 0.81 (3H,*d*, *J* = 6.7 Hz, H-27), 0.68 (3H,s,H-18); EIMS *m/z* (%): 576 $[M]^+$ (5), 414 $[M –Glc]^+$ (17), 399 $[M –Glc–Me]^+$ (15), 396 $[M –Glc–H_2O]^+$ (24), 381 (14), 329 (14), 303, 275, 273, 255

Bergapten (FL-**4)**: colorless needles; mp 188–189 °C (lit. mp 190–191°C); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ : 4.26 (3H, s, 4-OCH_3), 6.26 (1H, d, *J* =10.0 Hz, H-6), 7.01 (1H, dd, *J* = 1.0, 2.5 Hz, H-3), 7.12 (1H, dd, *J* = 0.5, 1.0 Hz, H-9), 7.59 (1H, d, *J* = 2.5 Hz, H-2), 8.14 (1H, dd, *J* = 0.5, 10.0 Hz, H-5); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 21.9 (-CH_3), 60.1 (-OCH_3), 105.0 (C-3), 106.4 (C-4a), 112.5 (C-6), 112.7 (C-3a), 139.2 (C-5), 144.8 (C-2), 149.6 (C-4), 152.7 (C-8a), 158.4 (C-9a), 161.1 (C-7).

Xanthotoxin (FL-**5)**: colorless needles; mp 146–147 °C (lit. mp 144–145 °C); ^{1}H-NMR ($CDCl_3$,300 MHz) δ: 4.29 (3H, s, -OCH_3), 6.35 (1H, d, *J* = 9.5 Hz, H-6), 6.82 (1H, d, *J* = 2.0 Hz, H-3), 7.34 (1H, s, H-4), 7.69 (1H, d, *J* = 2.0 Hz, H-2), 8.10 (1H, d, *J* =10.0 Hz, H-5); ^{13}C-NMR (CDCl3, 100 MHz) δ: 61.2 (-OCH_3), 107.7 (C-3), 112.9 (C-4), 114.7 (C-6), 116.5 (C-4a), 126.1 (C-3a), 132.8 (C-9),143.0 (C-8a), 144.3 (C-5), 146.6 (C-2), 147.7 (C-9a), 160.4 (C-7).

Scopoletin (FL-**6)**: light yellow needles; mp203–205 °C. (lit. mp 202–204 °C); ^{1}H-NMR ($CDCl_3$,300 MHz) δ: 3.78 (3H, s, 6-OCH_3), 6.30 (1H, d, *J*=9.5 Hz, H-3), 7.05 (1H, s, H-5), 7.11 (1H, s, H-8), 7.69 (1H, d, *J*=9.5 Hz, H-4); ^{13}C-NMR($CDCl_3$, 100 MHz) δ : 56.2 (-OCH_3), 104.1 (C-8), 109.6 (C-5), 111.1 (C-8a),112.4 (C-3), 116.5 (C-4a), 144.0 (C-4), 146.2 (C-6), 153.0 (C-7), 161.4 (C-2)

Isoimperatorin (FL-**7)**: light yellow prisms; mp 109–110 °C (lit. mp 108–110 °C); IR (KBr) ν_{max} cm^{-1}: 1721, 1587, 1146; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.72, 1.69 (6H, s, 3', 3'-CH3), 4.92 (2H, *J* = 7.1 Hz, H-1'), 7.54(1H, *J* = 7.1 Hz, H-2'), 6.26 (1H, d, *J*=10.0 Hz, H-6), 6.45 (1H, d, *J*=2.5 Hz, H-3),7.15 (1H, br s, H-9), 7.60 (1H, d, *J*=2.5 Hz, H-2), 8.16 (1H,d, *J*=10.0 Hz, H-5); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 18.2 (3'-CH_3), 25.8 (3'-CH_3), 69.7(C-1'), 94.1 (C-9), 105.0 (C-3), 107.4 (C-4a), 112.5 (C-6), 114.1 (C-3a), 119.0(C-2'), 139.6 (C-5), 139.8 (C-3'), 144.9 (C-1), 148.9 (C-4), 160.3 (C-2), 152.6 (C-8a), 158.1(C-9a), 161.3 (C-7).

Osthol (FL-8): Colorless needles; $C_{15}H_{16}O_3$; mp 80–81 °C (lit. mp 83–84 °C); ^{1}H-NMR ($CDCl_3$,300 MHz) δ: 1.67-1.84 (6H, s, 3', 3'-CH_3), 3.53 (2H, d, *J* = 7.5 Hz, H-1'), 3.91 (3H, br s, 7-OCH_3), 5.22 (1H, m, H-2'), 6.21 (1H, d, *J* = 9.5 Hz, H-3), 6.82 (1H, d, *J*=9.0Hz, H-6), 7.27 (1H, d, *J* = 9.0 Hz, H-5), 7.59 (1H, d, *J* = 9.5 Hz, H-4); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 17.9 (3'-CH_3), 21.9 (C-1'), 25.7 (3'-CH_3), 56.0 (7-OCH_3), 107.4 (C-6), 113.0 (C-3), 113.0 (C-4a), 121.1 (C-2'),126.2 (C-5), 132.6 (C-3'), 143.7 (C-4), 152.9 (C-8a), 160.2 (C-7), 97.2 (C-8),161.3 (C-2).

6,7-Dimethoxycoumarin (FL-9): $C_{11}H_{10}O_4$; mp 143-145 °C; IR (KBr) ν_{max} cm^{-1}: 3402, 1712,1610, 1496; EIMS 70 eV m/z (rel. int.): 206 $[M]^+$(100), 178 ($[M\text{-}CO]^+$ (94), 163 [M-CH_3-CO]+ (62), 135(35). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 7.96 (1H, d, J = 9.6 Hz, H-4), 6.41 (1H,br s, H-8), 6.29 (1H,br s, H-5), 6.15 (1H, d, J = 9.6 Hz, H-3), 3.89 (1H, s, 6 -OCH_3), 3.85 (1H, s, 7 -OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 164.4 (C-2), 162.2 (C-7), 157.6 (C-6), 157.5(C-8a), 139.4 (C-4), 111.6 (C-3), 104.7 (C-4a), 95.5 (C-5), 93.5 (C-8), 56.6 (7 -OCH_3), 56.5 (6 -OCH_3).

5, 7-dihydroxy-3', 4'-dimethoxy -6, 8-di (3-methylbut-2-enyl) flavanone (FL-10): yellow amorphous powder; $C_{27}H_{32}O_6$; UV λ_{max}: (MeOH) 290, 330; IR (KBr) ν_{max} cm^{-1}: 3384, 1603, 1450, 1630; Mass spectra m/z: 452 $[M]^+$, 434 $[M\text{-}H_2O]^+$, 397$[M\text{-}C_4H_7]^+$, 327[434-2C_4H_7]$^+$, 272, 288, 164, 55; ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm:12.76 (1H, br s, 5-OH), 7.01 (1H, d, *J* = 8.3, 1.9 Hz, 6′-H), 6.89 (1H, dd, *J* = 8.3 Hz, 5′-H), δ 6.76 (1H, d, *J* = 1.9 Hz, 2′-H), 6.40(1H, s, 6-OH), 5.51 (1H, dd, *J* = 2.8, 13.1 Hz, 2-H), 5.23(1H, d, *J*= 7.2 Hz, 2"- H), 5.18 (1H, d, *J*= 7.3 Hz, 2"'-H), 3.92 (3H, s, 5'-OCH_3), 3.88 (3H, s, 4'-OCH_3), 3.34 (2H, d, *J*= 6.4 Hz, 1"-H), 3.29 (2H, d, *J*= 6.8 Hz, 1"'-H), 3.11 (1H, dd, *J* = 13.1, 17.0 Hz, 3-H_α), 2.85 (1H, dd, *J* = 2.8, 17.0 Hz, 3-H_β), 1.70, 1.71, 1.81, 1.75 (3H each, s, 4"-OMe, 5"-OMe, 4"'-OMe, 5"'-OMe), ; ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C, ppm:196.7 (C-4), 162.4 (C-7), 160.1 (C-5), 157.0 (C-9), 149.4 (C-4'), 131.3 (C-1'), 113.1 (C-2'), 114.8 (C-5'), 121.7 (C-6'), 121.8 (C-2"), 149.6 (C-3'), 107.3 (C-6), 102.9 (C-10), 108.5 (C-8), 77.5 (C-2), 56.4 and 55.8 (3'/4'-OCH_3), 41.9 (C-3), 25.8 (C-5"), 21.3 (C-1"), 17.8 (C-4") 25.7 (C-5"'), 17.8 (C-4"'), 121.9 (C-2"') 22.0 (C-1"')

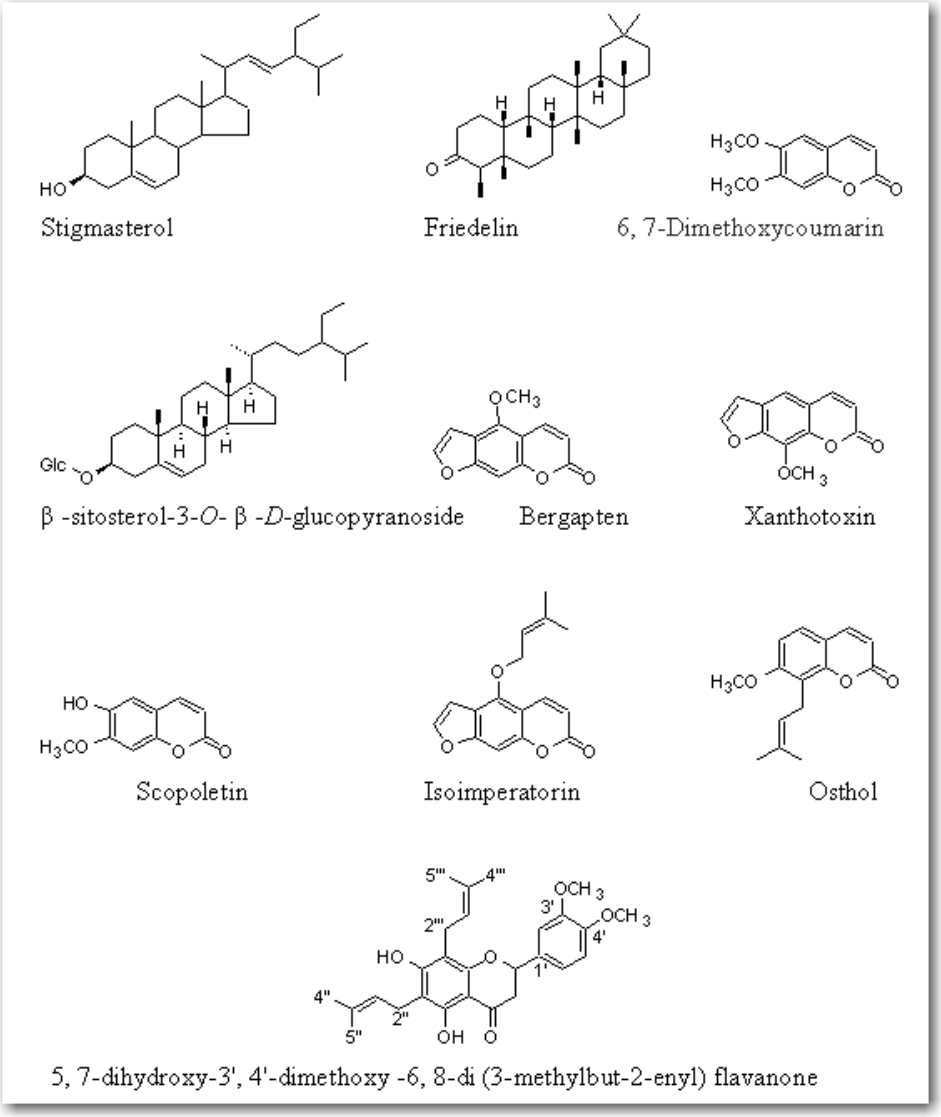
3. Result and discussion

The compound was isolated as yellow amorphous powder from ethyl acetate extract by eluting column with acetone: ethyl acetate (7:5). The MS of this compound give $[M]^+$ at *m/z* 452 corresponding to the molecular formula $C_{27}H_{32}O_6$. The IR spectrum of the compound showed the presence of hydroxyls (3384 cm^{-1}, br), aromatic groups (1603, 1450cm^{-1}) and a carbonyl group (1630 cm^{-1}). The UV spectrum of the compound illustrated bands 290 (4.00), 330 (3.45) nm. The IR and UV absorptions suggested the presence of a flavanone skeleton [19-21].

In the ^{1}H-NMR spectrum of the compound, there was one proton singlet at δ 12.36 being typical of C-5 hydroxyl group [19-21]. Moreover ^{1}H NMR also displayed signal of one other hydroxy group at δ 6.40 located at C-6. The assignment of C-7 was based on its HMBC correlations between 7-OH (δ_H 6.40) and C-6 (δ_C 107.3), C-7 (δ_C 162.4) and C-8 (δ_C 106.5). Besides, there was only one set of ABX system signals in the downfield region: δ 6.76 (1H, d, *J* = 1.9 Hz, 2′-H), 6.89 (1H, dd, *J* = 8.3 Hz, 5′-H) and 7.01 (1H, d, *J* =8.3, 1.9

Hz, 6′-H). In NMR two signals observed at δ_H 3.92 and δ_H 3.88 correlated with δ_C149.6 and δ_C 149.4 suggested the presence of two ortho substituted methoxy groups. The position of these two methoxy groups must be at C-3' and C-4'. Furthermore, there was another ABX system signals at δ 2.85 (1H, dd, *J* = 2.8, 17.0 Hz, 3-βH), 3.11 (1H, dd, *J* = 13.1, 17.0 Hz, 3-αH) and 5.51 (1H, dd, *J* = 2.8, 13.1 Hz, 2-H) in the ^{1}H-NMR [19-21].

The ^{13}C–NMR spectrum of the compound was corresponding with its ^{1}H-NMR, showing a carbonyl group signal at δ 196.7, C-2 and C-3 signals at δ 77.5 and 41.9 respectively. The ^{1}H and ^{13}C -NMR spectra of the compound also indicated the presence of two γ, γ-dimethyl allyl group (prenyl) moiety [22,23].



The ^{1}H-NMR spectrum showed the presence of two γ, γ-dimethyl allyl group at 1.70, 1.71, 1.81, 1.75 (3H each, s, Me-4", Me-5", Me-4"' and Me-5"'), 3.34 (2H, d, J= 6.4 Hz, H-1"), 3.29 (2H, d, J= 6.8 Hz, H-1"'), 5.23(1H, d, J= 7.2 Hz, H-2"), 5.18 (1H, d, J= 7.3 Hz, H-2"') which correlated to the ^{13}C NMR signals at 17.8 (C-4"), 25.8 (C-5"), 17.8 (C-4"'), 25.7(C-5"'), 21.3 (C-1"), 22.0 (C-1"'), 121.8 (C-2"), 121.9 (C-2"') respectively. In the HMBC spectrum correlations between H-1"/C-5, H-1"'/C-7 and C-8/C-9 suggested that one prenyl was linked to C-6 while the other linked on C-8 respectively [24-26].

Thus structure of this compound was assigned as 5, 7-dihydroxy-3', 4'-dimethoxy -6, 8-di (3-methylbut-2-enyl) flavanone. Several prenylated flavanone have been isolated from plants of various species [27], showed cytotoxic effect [27]. Survey of literature clearly indicated that this is a new prenyl flavanone [28,29].

Conclusion

We have successfully isolated 5, 7-dihydroxy-3', 4'-dimethoxy -6, 8-di (3-methylbut-2-enyl) flavanone from *Feronia limonia*. We believe this is the first report describing the isolation of 5, 7-dihydroxy-3', 4'-dimethoxy -6, 8-di (3-methylbut-2-enyl) flavanone from *Feronia limonia* and this compound is new from this plant.

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