



ISOLATION AND CHARACTERIZATION OF NOVEL COMPOUNDS FROM FRUITS OF *ZANTHOXYLUM LIMONELLA*

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ABSTRACT

The ethyl acetate fraction of methanolic extract of the fruits of *Zanthoxylum limonella* upon column chromatography resulted three compounds, Stigmast-5-en-3-ol-3-o-arbinopyranosyl-2'-deconate (z₁), β-sitosterol (z₂) and Stigmast-5-en-3-ol-3-o-rhamnopyranosyl-2'-octanoate (z₃). Among them, β-sitosterol is a well known phytoconstituent for its medicinal value, but relevant data of other two are unavailable.

Keywords: *Zanthoxylum limonella* fruits, Isolation, Characterization

INTRODUCTION

Zanthoxylum limonella is a deciduous and evergreen tree in Rutaceae family, native to warm temperate and sub-tropical areas worldwide. Various parts of *Z. limonella* are used as traditional medicine in India. The bark is bitter and used as a tonic to cure rheumatism. Essential oil extracted from the fruit is commonly known as 'Mullilam oil', frequently used as anti-inflammatory, anti-septic, anti-cholera, anti-diarrhoeal and hypo-cholesterolemic. Several alkaloids have been isolated from stem bark and one main compound isolated was leopol. Vitamin E and aromatic components have been isolated from seed oil¹⁻⁷.

MATERIALS AND METHODS

Soxhlation was conducted by using Borosil soxhlet extractor. Melting points were determined on Labard melting point apparatus, FTIR spectra were recorded on Spectrum 400, Perkin Elmer. UV spectra were measured on UV-visible spectrophotometer UV-1800, Shimadzu. ¹H NMR was recorded on Bruker avance II 400 NMR spectrophotometer in solvents CDCl₃ and DMSO. Mass spectra were obtained on a TOF MS instrument.

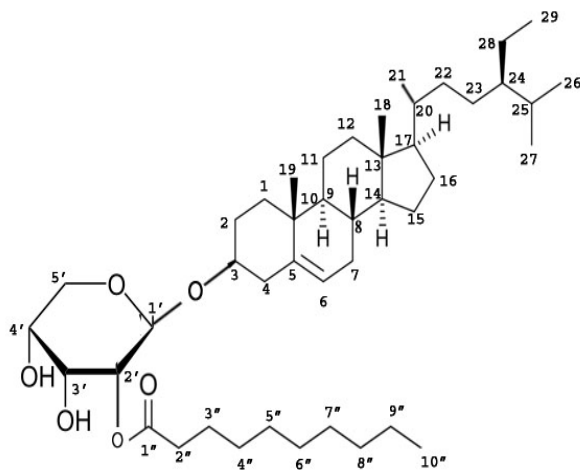
Isolation and Characterization

Fruits of *Zanthoxylum limonella* was purchased from local market in Kerala, India and authenticated by NISCAIR [authentication number – 1283/87]. 3 Kg of the coarse powdered drug was used for the extraction. Following general method (page no. 268) was employed⁸⁻¹². The total yield of extract was 69 g for 3 kg of the powder. The extract was subjected to column chromatography and elution was carried out successfully with 100 ml portions, each of toluene along with graded mixtures of solvents in a ratio like toluene:chloroform (95:5, 90:10, 85:15 up to 100 %

chloroform). Similar graded mixtures of chloroform:ethyl acetate and ethyl acetate: methanol were employed¹³⁻¹⁵. The fractions were combined based on their TLC pattern and individual compounds were further purified by preparative thin layer chromatography on silica gel G (for TLC)¹⁶⁻¹⁹ in the presence of chloroform:methanol (9:1, 8:2, 7:3 and chloroform 100 %) as mobile phase; afforded three compounds namely Stigmast-5-en-3-ol-3-o-arbinopyranosyl-2'-deconate(z₁), β-sitosterol (z₂) and sitosteryl rhamnosyl caprylate(z₃).20-21

RESULTS AND DISCUSSION

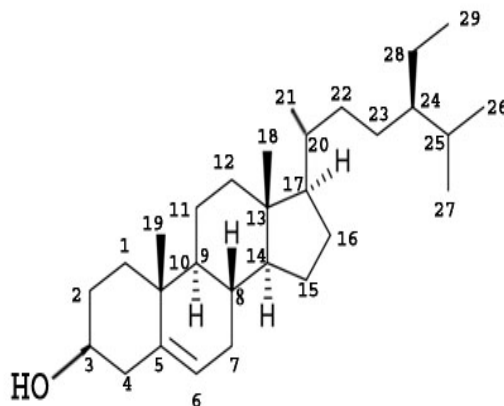
Compound z₁ (Stigmast - 5en-3-ol-3-o-arbinopyranosyl -2'- deconate): Red colored crystalline substance; R_f Value 0.82 in chloroform : methanol (9:1); m.p. 80-85⁰C; UV λ_{max} 381 nm (methanol); IR ν_{max} (KBr): 3435, 2926, 2855, 1734, 1639, 1457, 1378, 1247, 1169, 1080, 1039, 722 cm⁻¹; ¹HNMR (CDCl₃, 400MHz): δ 5.48 (1H, m, H-6), 5.44(1H, brm, H-1'), 4.64-3.98 (3×1H, m, H-2', H-3', H-4'), 4.14 (1H, m, 4¹-OH), 3.80 (1H, d, J=7.06Hz, 3¹-OH), 3.71(1H, brm, H-3), 3.59 (2H, t, J = 7.2Hz, H₂-5'), 2.67 (3H, brs, H-8, H-9, H-14), 2.59-1.67 (12H, 6×CH₂, H₂-1, H₂-2, H₂-4, H₂-7, H₂-11, H₂-12), 2.46 (3H, m, H-20, H-24, H-25), 2.42 (1H, dd, J = 1.98, 4.12Hz, H-17), 2.24 (2H, t, J = 7.2Hz, CH₂-2"), 1.46 (4H, brm, H₂-15, H₂-16), 1.37 (6H, m, H₂- 22, H₂-23, H₂-28), 1.26 (14H, brs, 8×CH₂, H₂-3", H₂-4", H₂-5", H₂-6", H₂-7", H₂-8", H₂-9"), 1.18 (3H, brs, Me-19), 1.02 (3H, d, J = 6.5Hz, Me-21), 0.89 (3H, d, J = 6.5 Hz, Me- 26), 0.84 (3H, d, J = 6.1Hz, Me-27), 0.80 (3, d, J = 6.3Hz, Me-29), 0.75 (3H, brs, Me-18), 0.71 (3H, t, J = 7.1Hz, Me-10"); LC – MS m/z (rel.int) :700 [M]⁺ (C₄₄H₇₆O₆) (7.4), 685 (17.9), 663 (9.7), 639 (6.4), 595 (7.5),551 (10.3), 507 (10.1), 463 (11.2), 413 (51.0), 367 (13.1), 349 (47.9), 333 (100), 288 (29.6), 279 (8.1), 133 (5.2).



Compound z₁
(Stigmast-5-en-3-ol-3-o-arbino pyranosyl -2'-deconate)

Compound z₂ (Stigmast - 5-en-3-ol) (β- sitosterol): Red colored crystalline substance; R_f Value 0.5 in chloroform : methanol (9:1); m.p.144-147^oC; 297 UV λ_{max} nm (methanol); IR ν_{max} (KBr): 3433, 2925, 2854, 2090, 1640,1553, 1458, 1404, 1080, 667 cm⁻¹; ¹HNMR (DMSO-d₆, 400MH_z): δ 5.25 (1H, m, H-6), 3.52 (1H, brm, W_{1/2}18.4H_z, H-3), 2.91 (1H, OH), 2.88 (3H, brs, H-8, H-9, H-14), 2.87-1.51 (12H, 6×CH₂, H₂-1, H₂-2, H₂-4, H₂-7, H₂-11, H₂-12), 2.28 (3H, m, H-20, H-24, H-25), 2.02 (1H, dd, J=1.98,

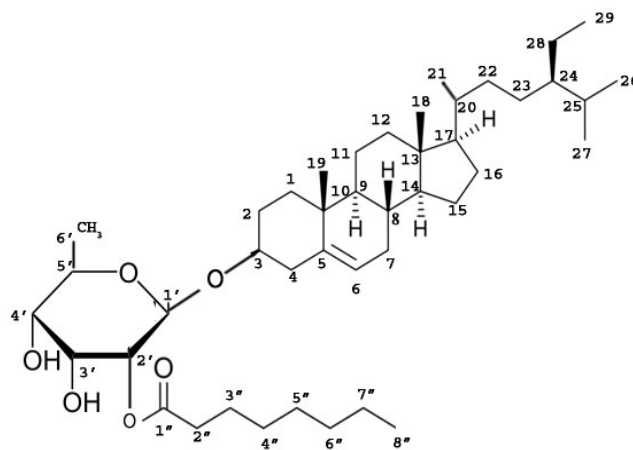
4.12H_z, H-17), 1.51 (4H, brm, H₂-15, H₂-16), 1.39 (6H, m H₂-22, H₂-23, H₂-28), 1.05 (3H, brs, Me-19), 0.95 (3H, d, J=6.2H_z, Me-21), 0.89 (3H, d, J=6.6H_z, Me-26), 0.82 (3H, d, J=6.5H_z, Me-27), 0.80 (3H, d, J=6.1H_z, Me-29), 0.71(3H, brs, Me-18); LC – MS m/z (rel.int): 414 [M]⁺ (C₂₉H₅₀O) (41.2),408 (9.4),403 (11.8), 395 (13.5),390 (41.6), 382 (9.0), 376 (46.6), 367 (42.9),360 (100), 351 (23.9),346 (75.9), 333 (29.6), 328 (16.9), 292 (34.8), 253 (16.6), 212 (8.2),127 (30.0),.



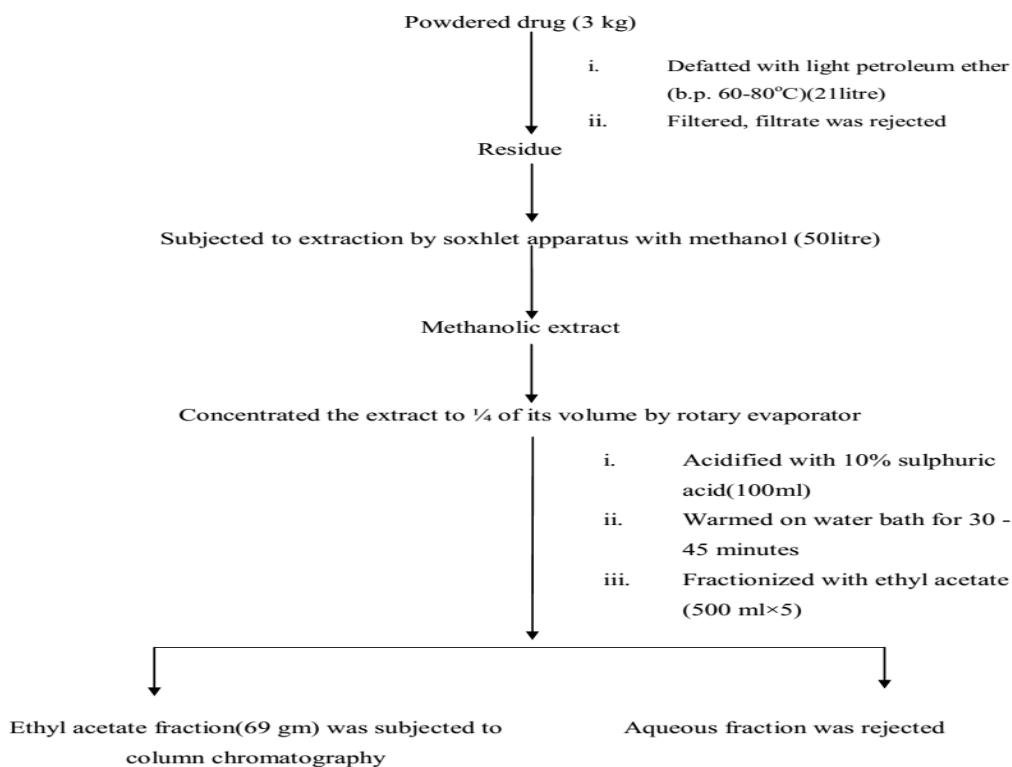
Compound z₂
(Stigmast-5-en-3-ol) (β- sitosterol)

Compound z₃ (Stigmast-5-en-3-ol-3-o-rhamnopyranosyl - 2'-octanoate): Blue colored crystalline substance; R_f Value 0.82 in 100 % CHCl₃; m.p.102-107^oC; UV λ_{max} 234 nm (methanol); IR ν_{max} (KBr): 3431, 2921, 2851, 1740, 1631, 1462, 1377, 1080, 774, 463 cm⁻¹; ¹HNMR (DMSO-d₆,400MH_z): δ 5.45 (1H,m, H-6), 5.27 (1H, brm, H-1'), 4.53(1H, m, 4'-OH), 4.5-3.80 (4×1H, m , H-2', H-3', H-4', H-5''), 3.80 (1H,D, J=7.06H_z, 3'-OH), 3.66 (1H,brm,H-3), 2.63 (3H, brs, H-8, H-9, H-14), 2.51-1.61 (12H,6 × CH₂, H₂-1, H₂-2, H₂-4, H₂-7, H₂-11, H₂-12), 2.50 (3H, m, H-20, H-24, H-25), 2.41 (1H,dd, J=1.98, 4.12H_z, H-17), 2.30 (2H, t, J=7.1H_z, H₂-2''), 1.48 (4H, brm, H₂-15, H₂-16), 1.35 (6H, m,

H₂-22, H₂-23, H₂-28), 1.21 (10H, brs, 5×CH₂, H₂-3'', H₂-4'', H₂-5'', H₂-6'', H₂-7''), 1.16 (3H, brs, Me-19), 1.02 (3H, d, J=6.1H_z, Me-21), 0.90 (3H, d, J=6.6H_z, Me-26), 0.85 (3H,d, J=6.4H_z, Me-7), 0.80 (3H, d, J=6.5H_z, Me-29), 0.76 (3H, brs, Me-18), 0.68 (3H, t, J=7.3H_z, Me-8''), 0.78 (3H, brs, Me-6''); LC–MS m/z (rel.int): 686[M]⁺ (C₄₃H₇₄O₆) (14.3), 663(11.5), 640(9.0), 633 (10.4), 619(8.1), 605(9.2), 595(23.27), 575(8.2), 551(29.2), 531(10.7), 507(28.07), 487(13.9), 463(28.6), 449(6.6), 437(9.4), 413(100), 399(13.8), 375(15.3), 360(46.0), 331(9.8), 301(86.1), 274(15.1), 169(4.5), 149(8.8), 117(5.7).



Compound z3
(Stigmast-5-en-3-ol-3-o-rhamno pyranosyl-2'-octanoate)



CONCLUSION

Upon characterization with the help of spectral data proved the presence of β - sitosterol, Stigmast-5en-3-ol-3-o-arbinopyr- anosyl-2'-deconate and Stigmast-5-en-3-ol-3-o-rhamno pyranosyl -2'-octanoate. They are being first reported from *Zanthoxylum limonella* fruits, in which β - sitosterol is common in many plants. The other two compounds seem to be esters of sitosterol but relevant data are unavailable. However, other spectral studies like ^{13}C NMR and 2D experiments (COSY NMR) are essential to confirm structure of these compounds.


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