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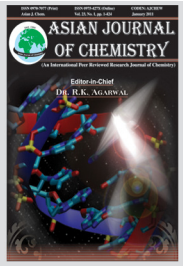

Analysis of Precipitates from Acetone Extracts of *Feronia limonia*

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Vitexin and sucrose were isolated from acetone extracts of air-dried flowers of *Feronia limonia*. Their structures were elucidated by spectral studies and comparison with literature data.

Key Words: Vitexin, Sucrose, *Feronia limonia*.

INTRODUCTION

Rutaceae, a major plant family, is a good source of edible fruits and rich in essential oils, alkaloids, coumarins and flavonoids. Different parts of this plant family are used as folk medicines[1] in West Africa. The wide spectrum of biological uses of these plants led to the isolation of several components from their aerial parts. Varieties of indoloquinolines, acridone alkaloids such as acronycine, glyfoline and benzo fused acronycines are isolates of *Rutaceae* and are used as DNA intercalators[2-5]. Also some furo, pyrano and linear quinoline alkaloids were isolated from the *Rutaceae* family and were reported as antimalarial, antibacterial, antiviral, antitrypnocidal and antileishmaniasis[6-8].

Although various methods are available to synthesize these alkaloids[9-13], phytochemists are continuously exploring the potential of new plants; compounds with better efficacy against diseases[14-17] are being isolated. Our reports on the plant families Meliaceae[18,19] and Fabeaceae[20] contributed substantial information on phytochemistry.

In continuous search for physiologically active compounds in plants, we investigated the chemical constituents of *Feronia limonia*, belonging to the *Rutaceae* family, a commonly occurring tropical plant species in the Indian subcontinent[21]. Limited reports are documented on the phytochemistry and pharmacology of this species[22,23]. Isolated constituents from the root bark are coumarins, steroids, alkaloids, fatty acids, aromatic hydrocarbons and glycosides[21]. An acidic polysaccharide was also isolated from the fruit and showed good activities against tumor cells[22]. Compounds isolated from the root and stem bark are stigmasterol, friedelin, β-sitosterol-3-O-β-D-glucopyranoside, bergapten, xanthotoxin, scopoletin, isoimperatorin, osthol and 6,7-dimethoxycoumarin[23].

During our investigation with flowers of the plant, we were surprised to observe some precipitates in the acetone extract, which was unexplored at least to the best of our knowledge. In this study, the details of isolation and structure elucidation of the two isolates are reported.

EXPERIMENTAL

Melting points were determined on an electrically heated melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Paragon 1000 FTIR spectrophotometer as potassium bromide discs unless otherwise indicated. ^{1}H NMR spectra were obtained on a Brucker (500 MHz) instrument in either D_2O or DMSO-d_6 solutions using tetramethylsilane as an internal standard. *J* values are given in Hz. Thin layer chromatography were run on glass plates coated with silica gel G and visualized using iodine vapour. All the basic chemicals and solvents were purchased from Rankem (India).

The plant materials were collected during April 2005 from Perundurai, Erode District, Tamil Nadu, India and identified by Dr. Geetha, Department of Botany, Government College, Tiruppur, Tamilnadu, India.

De-Waxing of flower: The fresh flowers were dried under shade at room temperature for 15 days. The dried flowers (2.5 kg) were chopped, extracted with *n*-hexane and left overnight. The solvent was filtered, collected, concentrated in a rotary evaporator and the waxy material was discarded.

Extraction and isolation: Wet flowers were air-dried for 3 h to remove excess hexane and then extracted with acetone in a Soxhlet apparatus for 5 days. The acetone extract was allowed to stand for slow evaporation at room temperature; a mixed yellow solid and a white crystalline compound, which gradually separated out, were filtered and repeatedly washed

with acetone. Thin layer chromatography (TLC) indicated the presence of a mixture of two compounds; white cubic crystals and a yellow solid.

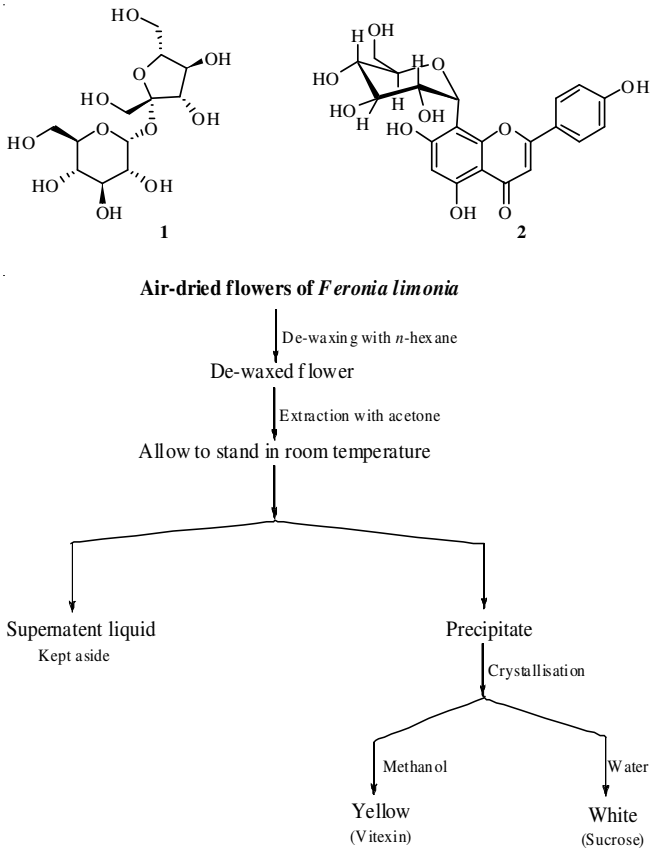
Compound 1: It was crystallized from water and melted at 186 ºC. It gave a burning sugar smell on heating (charring); resulted in a positive Molish test and negative Fehling and Tollen's tests. On warming with dilute HCl, it hydrolyzed to glucose and fructose; the resultant mixture reduced Fehling solution and gave positive Molish test. It was concluded that the compound was a disaccharide and a non-reducing sugar. The spectral data exactly matched with an authentic sample of sucrose[24].

Compound 2: The mixture was washed with methanol and recrystallised to produce yellow needles Yield: 45 mg; m.p. 272 ºC; UV (MeOH, λ_{max}, nm): 269, 302, 332; IR (KBr, ν_{max}, cm^{-1}): 3380, 1654, 1616, 1568, 1504, 1365, 1293, 1099, 1044; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.15 (s, 1H, 5-OH), 8.01 (d, 2H, J = 8.5 Hz, $C_{2'}$- H and $C_{6'}$-H), 6.90 (d, 2H, J = 8.5 Hz, $C_{3'}$-H and $C_{5'}$-H), 6.77 (s, 1H, C_3-H), 6.26 (s, 1H, C_6-H), 4.68 (d, 1H, J = 10 Hz, $C_{1''}$-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: C_4-182.5, C_2-163.5, C_7-162.5, $C_{4'}$-161.5, C_9-160.8, C_5-156.4, $C_{2'}$ and $C_{6'}$-129.4, $C_{1'}$-122.1, $C_{3'}$ and $C_{5'}$-116.2, C_8-105.0, C_{10}-104.4, C_3-102.9, C_6-98.6, $C_{5''}$-82.3, $C_{1''}$-79.1, $C_{2''}$-73.8, $C_{3''}$-71.2, $C_{4''}$-71.0, $C_{6''}$-61.7.

RESULTS AND DISCUSSION

The precipitates from the acetone extract of *Feronia limonia*, **Scheme-I** yielded two compounds. Compound **1** was isolated as white crystals by washing the mixture followed by recrystallization with water. The cubic crystals melted at 186 ºC, became charred upon heating and gave a burning sugar smell. The Molish test for the presence of carbohydrates gave a postive result whereas the Fehling's and Tollen's tests gave negative result thereby indicating an acetal functional group. By comparing the spectral data with literature data, compound **1** was identified as sucrose.

Compound **2** was obtained as a yellow solid after recrystallization with methanol. It melted at 270 ºC and elemental analysis corresponded to the formula $C_{21}H_{14}O_{10}$. Under UV light **2** fluoresced yellow; it gave a characteristic red colour with Shinoda's test thereby indicating a flavonoid and also a brownish colour with alcoholic ferric chloride indicating a chelated hydroxyl group. It showed two absorptions at λ_{max} (MeOH) 265 and 335 nm in UV spectrum. Its IR spectrum showed absorption bands at 3380 and 1654 cm^{-1} ascribable to phenolic O-H and C=O stretching of flavone[25,26]. The ^{1}H NMR spectrum showed a downfield resonance at δ 13.1 which was attributed to a chelated hydroxyl proton and it was exchangeable with D_2O. ^{1}H NMR data indicated the presence of AA'BB' pattern in ring B, suggesting that the flavone moiety in compound **2** was apigenin (Apigenin is a flavone that is the aglycone of several glycosides. It is a yellow crystalline solid that has been used to dye wool). ^{13}C NMR spectrum revealed twenty one carbon signals, which suggested a flavonoid and a saccharide moiety. The six carbon signals characteristic of a sugar moiety were present at δ 82.30 ($C_{5''}$) , 79.11 ($C_{1''}$), 73.83 ($C_{2''}$), 71.29 ($C_{3''}$), 70.98 ($C_{4''}$) and 61.74 ($C_{6''}$) thereby suggesting compound **2** is a flavone C-glycoside[27]. The sugar moiety was



Scheme-I

determined to be at the C_8 position of the aglycon moiety. The glycosidation position was unambiguously determined at the C_8 position by the appearance of glucosyl anomeric proton $C_{1''}$-H at δ 4.68 (d, J = 10 Hz). Based on these data, compound **2** was identified as apigenin 8-C-β-D glucopyranoside (vitexin). The data was compared with reported values[28] and it was found to be identical in all aspects. The occurrence of vitexin is reported for the first time from this plant source.

Conclusion

The precipitates obtained from the acetone extract of the flowers of *Feronia limonia* yielded two compounds. They were identified as sucrose and vitexin.

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REFERENCES

1. (a) D.H. Kim, E.A. Bae and M.J. Han, *Biol. Pharm. Bull*., **22**, 422 (1999); (b) H.M. Kim, H.J. Kim and S.T. Park, *J. Ethnopharmacol*., **66**, 283 (1999); (c) H. Fujioka, Y. Nishiyama, H. Furukawa and N. Kumada, *Antimicrob. Agents Chemother*., **33**, 6 (1989); (d) N. Yamamoto, H. Furukawa, Y. Ito, S.Yoshida, K. Maeno and Y. Nishiyama, *Antiviral Res*., **12**, 21 (1989); (e) S.F. Queener, H. Fujioka, Y. Nishiyama, H. Furukawa, M.S. Barlett and J.W. Smith, *Antimicrob. Agents Chemother*., **35**, 377 (1991).

2. (a) W. Peczyriskc-Czoch, F. Pognan, L. Kaczmarck and J. Boratyriski, *J. Med. Chem*., **37**, 3503 (1994); (b) G.S.M. Sundaram, C. Venkatesh, U.K.S. Kumar, H. Ila and H. Junjappa, *J. Org. Chem*., **69**, 5760 (2004).
3. W.D. Wilson and R. Jones, In ed.: A.J. Jacobson, Intercalation Chemistry, M.S. Whittinham, Academic Press, New York, Ch. 14 (1981).
4. (a) A. Molina, J.J. Vaquero, J.L. Garcia-Navio, J. Alvarez-Builla, B. Pascula-Terasa, F. Gago, M.M. Rodrigo and M. Ballestros, *J. Org. Chem*., **61**, 5587 (1996); (b) K. Cimanga, T. DeBruyne, L. Pieters, A.J. Vlietinck and C.A. Turger, *J. Nat. Prod*., **60**, 688 (1997); (c) A. Paulo, E.T. Gomes, J. Steele, D.C. Warhurst and P.J. Houghton, *Planta Med*., **66**, 30 (2000); (d) S.V. Miert, S. Hostyn, B.U.M. Maes, K. Cimanga, R. Brun, M. Kaiser, P. Matyus, R. Dommisse, G. Lemiere, A. Vlietinck and L. Pieters, *J. Nat. Prod*., **68**, 674 (2005).
5. S. Michel, T. Gaslonde and F. Tillequin, *Eur. J. Med. Chem*., **39**, 649 (2004).
6. (a) D. Karou, A. Savadogo, A. Canini, S .Yameogo, C. Montesano, J. Simpore, V. Colizzi and A.S. Traore, *Afr. J. Biotechnol*., **4**, 1452 (2005); (b) B.E.P.O. Bever, Medicinal Plants in Tropical West Africa, Cambridge University Press, Cambridge (1986); (c) K.Q.J. Boakye-Yiadom, *Zrude Drug Res*., **17**, 78 (1979).
7. A.R.P. Ambrozin, J. Mafezoli, P.C. Vieira, J.B. Fernandes, M.F.G.F. da Silva, J.A. Ellen and S. Albuquerque, *J. Braz. Chem. Soc*., **16**, 434 (2005).
8. A. Fournet, A.A. Barrios, V. Munoz, R. Hoquemiller, A. Cave and J. Bruneton, *Antimicrob Agents Chemother*., **31**, 859 (1993).
9. P. Pitchai, P.S. Mohan and R.M. Gengan, *Indian J. Chem. B. Org. Chem. Incl. Med. Chem*., **48B**, 692 (2009).
10. T. Dhanabal, R. Sangeetha and P.S. Mohan, *Tetrahedron Lett*., **46**, 4509 (2005).
11. R. Nandhakumar, T. Suresh and P.S. Mohan, *Tetrahedron Lett*., **43**, 3327 (2002).
12. T. Dhanabal, R. Sangeetha and P.S. Mohan, *Tetrahedron*, **62**, 6258 (2006).
13. T. Dhanabal, R. Sangeetha, R.M. Gengan and P.S. Mohan, *J. Chem. Res*., **2**, 124 (2007).
14. I. Kuzovkina, I. Al'terman and B. Schneider, *Phytochemistry*, **65**, 1095 (2004).
15. G. Jayakumar, M.D. Ajthabai, B. Harikumar, P.K. Viswanathan, B. Remeshkumar and G. Rajkumar, *Indian J. Chem. B. Org. Chem. Incl. Med. Chem*., **49B**, 112 (2010).
16. C.H. Park, T.W. Kim, S.H. Son, J.Y. Hwang, S.C. Lee, S.C. Chang, S.H. Kim, S.W. Kim and S.K. Kim, *Phytochemistry*, **71**, 380 (2010).
17. M.L.R. Ferreira, I.C. de Pascoli, I.R. Nascimento, J.Z. Schpector and L.M. Lopes, *Phytochemistry*, **71**, 469 (2010).
18. S.T. Selvi and P.S. Mohan, *Indian J. Chem. B. Org. Chem. Incl. Med. Chem*., **36B**, 1082 (1997).
19. C. Balasubramanian, P.S. Mohan, K. Arumugasamy and K. Udaiyan, *Phytochemistry*, **34**, 1194 (1993).
20. P. Siddhuraju, P.S. Mohan and K. Becker, *Food Chem*., **79**, 61 (2002).
21. A.A. Rahuman, G. Gopalakrishnan, B.S. Ghouse, S. Arumugam and B. Himalayan, *Fitoterapia*, **71**, 553 (2000).
22. Y. Saima, A.K. Das, K.K. Sarkar, A.K. Sen and P. Sur, *Int. J. Biol. Macromol*., **27**, 333 (2000).
23. J. Intekhab and M. Aslam, *Anal. Univ. Bucuresti-Chim. (serie noua)*, **18**, 95 (2009).
24. D. Rinaldo, C.M. Rodrigues, J. Rodrigues, M. Sannomiya, L.C. dos Santos and W. Vilegas, *J. Braz. Chem. Soc*., **18**, 1132 (2007).
25. Y.K. He and H.R. Dong, Handbook of Analytical Chemistry, Chemical Industry Press, Beijing, edn. 2, p. 655 (1998).
26. K.R. Markham, Techniques of Flavonoid Identification, Academic Press, London, p. 81 (1982).
27. M. Tomczyk, J. Gudeja and M. Sochacki, *Z. Naturfortsh*, **57c**, 440 (2002).
28. A. De Bruyn and J. Van Loo, *Carbohydr. Res*., **211**, 131 (1991).