

Triterpenes from the Root Bark of *Phyllanthus Columnaris*

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Abstract: Phytochemical study on the root bark of *Phyllanthus columnaris* obtain from Paula Langkawi has been conducted. The separations of the chemical components were carried out by different chromatography techniques and their structures were elucidated by spectroscopic methods such as mass spectrometry (MS), ¹H and ¹³C NMR and by comparison with those of previously reported data. Three triterpenes were isolated which were identified as stigmasterol, taraxerone and taraxerol.

Key words: Euphorbiaceae, *Phyllanthus columnaris*, stigmasterol, taraxerone and taraxerol.

INTRODUCTION

Plants of the genus *Phyllanthus* are part of the Euphorbiaceae family. Comprising more than 500 species, *Phyllanthus* are widely distributed throughout South Africa and Asia. Many of them are used medicinally in different countries. *Phyllanthus columnaris* is a not a big tree up to 7 m high that grows luxuriantly in Perlis and Langkawi Agharkar, 1991. Its roots, leaves, fruits, milky juice and whole plants are used as medicine Whitmore, 1972. Fruits are useful for tubercular ulcers, wounds, sores, scabies and ring worm Kirtikar and Basu, 1935.. In this paper, we report the isolation and characterization of three known compounds from *Phyllanthus columnaris* namely.) stigmasterol, taraxerone and taraxerol.

MATERIALS AND METHODS

General Experimental Procedure:

Melting points were measured on Gallenkamp apparatus and were uncorrected. IR spectra were obtained with CHCl₃ as a solvent on a Perkin-Elmer FT-IR 1725-X. 1H (400 MHz) and 13C (100 MHz) measurements were carried out on a JEOL ECP-400 spectrometer. Chemical shifts are reported in ppm and the coupling constants are given in Hz.

Plant material:

The bark roots of *Phyllanthus columnaris*, which were collected from pula Langkawi Voucher specimens of WAY131 have been deposited at the Herbarium of Universiti Kebangsaan Malaysia.

Extraction and Isolation:

The dried powdered root bark (300 g) of *P. columnaris* was macerated in MeOH at room temperature for 72 hours to give, after solvent evaporation, a dark brown gum of MeOH extract (2.5 g). the MeOH was then subjected to vacuum liquid chromatography (VLC) eluted with Hexane, Hexane-EtOAc of increasing polarity to give three sub fractions each fraction were repeated chromatography omit, Hexane radial chromatography and eluted Hexane: EtOAc of increasing polarity, yield compound 1 (2.5 mg), compound 2 (3.4) and compound omit; n-hexane 3 (4.7 mg).

Stigmasterol 1:

Was obtained as a white powder. m.p. 165 °C; IR (CHCl₃): ν_{\max} = 3433, 2923, 1638, 1456, 1374, 1221, 894. MS: 412 [M⁺], 351, 314, 300, 271, 229, 213 cm⁻¹. 1H NMR (CDCl₃, 400 MHz): δ 5.36 (1H, d, *J* =

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4.8Hz, H-6), 5.15 (1H, dd, $J = 8.8, 15.4$ Hz, H-22), 5.01 (1H, dd, $J = 8.8, 14.8$ Hz, H-23), 3.53 (1H, m, H-3), 1.02 (3H, s, H-19), 0.92 (3H, d, $J = 6.6$ Hz, H-21), 0.86 (3H, d, $J = 7.7$ Hz, H-26), 0.82 (3H, t, $J = 7.3$ Hz, H-29), 0.79 (3H, d, $J = 8.4$ Hz, H-27), 0.68 (3H, s, H-18). ^{13}C NMR (CDCl_3 , 100 MHz): δ 140.9 (C-5), 138.5 (C-22), 129.5 (C-3), 121.9 (C-6), 72.0 (C-3), 57.0 (C-14), 56.1 (C-17), 51.4 (C-24), 50.3 (C-9), 46.0 (C-25), 42.4 (C-13), 40.7 (C-20), 39.8 (C-12), 37.5 (C-4), 37.4 (C-1), 36.7 (C-10), 32.1 (C-8), 31.9 (C-7), 29.2 (C-16), 28.4 (C-2), 25.6 (C-28), 24.5 (C-15), 21.4 (C-21), 21.3 (C-11), 20.0 (C-27), 19.6 (C-26), 19.1 (C-19), 12.2 (C-29), 12.1 (C-18).

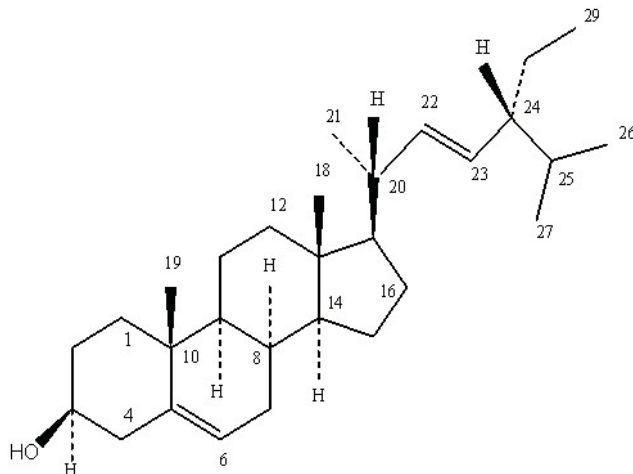


Fig. 1

Taraxerone 2

Was obtained as a Colourless needles (3.4 mg), m.p 240°C; IR (CH_3Cl): $\nu_{\text{max}} = 2960, 1718, 1458, 1374$ cm^{-1} . ^1H NMR (CDCl_3 , 400 MHz): δ_{H} 5.56 (1H, d, $J = 4.7$ Hz, H-15), 1.14(3H, s, H-27), 1.09 (3H, s, H-23), 1.08 (3H, s, H-25), 1.07 (3H, s, H-24), 0.96 (3H, s, H-29), 0.92 (3H, s, H-28), 0.91 (3H, s, H-30), 0.83 (3H, s, H-26). ^{13}C NMR (CDCl_3 , 100 MHz): δ_{C} 217 (C-3), 157.8 (C-14), 117.4 (C-15), 56.0 (C-5), 49.0 (C-18), 48.9 (C-9), 47.8 (C-4), 40.8 (C-19), 39.1 (C-8), 38.5 (C-1), 37.9 (C-10/17), 37.8 (C-13), 36.9 (C-16), 36.0 (C-12), 35.3 (C-7), 34.4 (C-2), 33.8 (C-21), 33.6 (C-29), 33.3 (C-22), 30.2 (C-28), 30.1 (C-26), 29.0 (C-20), 26.3 (C-23), 25.8 (C-27), 21.7 (C-24), 20.2(C-6), 17.7 (C-11), 15.0 (C-25). MS m/z (int. rel.): 424 [M^+], 409, 300, 204.

Taraxerol 3

Was obtained as a Colourless powder (4.7 mg), m.p 139-140°C; IR (CH_3Cl): $\nu_{\text{max}} = 3392, 2929, 1611, 1513, 1454, 1385$ cm^{-1} . MS m/z (int. rel.): 426 [M^+], 409, 302, 287, 218, 204, 189, 135. ^1H NMR (CDCl_3 , 400 MHz): δ_{H} 5.53 (H-15), 3.20 (H-3), 1.09 (H-27), 0.98 (H-23), 0.96 (H-29), 0.93 (H-24), 0.91 (H-26/30), 0.83 (H-28), 0.81 (H-25). ^{13}C NMR (CDCl_3 , 100 MHz): δ_{C} 158.3 (C-14), 117.1 (C-15), 79.3 (C-3), 55.7 (C-5), 49.5 (C-18), 48.9 (C-9), 41.5 (C-19), 39.2 (C-8), 39.0 (C-4), 38.2 (C-1), 37.9 (C-10/13/17), 36.9 (C-16), 36.0 (C-12), 35.3 (C-7), 33.9 (C-21), 33.6 (C-29), 33.3 (C-22), 30.1 (C-26), 30.0 (C-28), 29.0 (C-20), 28.2 (C-23), 27.4 (C-2), 26.1 (C-27), 21.5 (C-30), 19.0 (C-6), 17.7 (C-11), 16.0 (C-25), 15.6 (C-24).

RESULTS AND DISCUSSION

Three compounds were successfully isolated from the root bark of *P. columnaris*.

Compound 1:

was obtained as white powder with m.p. 165 °C. The mass spectrum (Ms) exhibited a molecular ion peak at m/z 412 which consistent with the formula molecule $\text{C}_{29}\text{H}_{48}\text{O}$. The IR spectrum showed a broad band at 3392 cm^{-1} indicated the presence of O-H. Additional band at 1638 cm^{-1} were typical of non-conjugated alkene. The ^1H NMR spectrum displayed a multiplet at δ 3.53 corresponding to proton (H-3). Three olefinic protons

in which the last two are of *trans* configuration were observed at δ 5.36 (1H, d, $J = 4.8$ Hz), 5.15 (1H, dd, $J = 8.8, 15.4$ Hz) and 5.01 (1H, dd, $J = 8.8, 14.8$ Hz) ascribable to H-6, H-22 and H-23 respectively. The APT NMR spectrum of compound 1 exhibited 29 signals. Signals at δ 140.9, 138.5, 129.5 and 121.9 were observed for four olefinic carbons, corresponding to C-5, C-6, C-22 and C-23 respectively. Signal for six methyl carbons (C-18, C-29, C-19, C-26, C-27 and C-21) could be seen in the most upfield region of the NMR spectrum at δ 12.1, 12.2, 19.1, 19.6, 20.0 and 21.4 respectively. In addition signal for the carbon C-3 which carried the hydroxyl group appeared at δ 72.0.

Stigmasterol has been reported to occur in *Tephrosia pumila* Genapaty *et al.*, (2008), *Ferula diversivittata* Iranshahi Mehrdad *et al.*, 2008. and *Withania somnifera* Misra Laxminarain *et al.*, 2008.

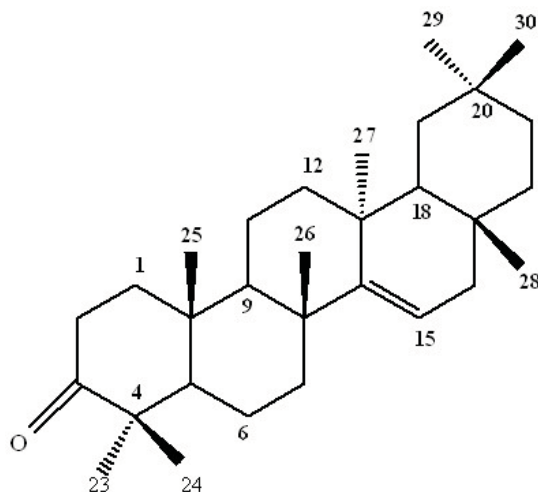


Fig. 2

Compound 2:

was obtained as Colourless needles with m.p. 240 °C. The mass spectrum (Ms) exhibited a molecular ion peak at m/z 424 which consistent with the formula molecule $C_{30}H_{48}O$. The 1H NMR spectrum showed eight singlet signals representing eight methyl groups at δ 0.83, 0.91, 0.92, 0.96, 1.07, 1.08, 1.09 and 1.14 for H26, H30, H28, H29, H24, H25, H23 and H27 respectively. The presence of all the methyl groups as singlet supported the fact that all methyl groups in Taraxerone are attached to quaternary carbons. Doublet signal appeared at δ 5.56 with coupling constant of 4.7 Hz for one olefinic proton at H15 coupled with the protons of H16. The ^{13}C NMR of the compound 2 showed 30 signals which was represented by eight methyl groups. A carbonyl was observed at δ 217.9. The double bond was represented by two singlets at δ 157.8 and 117.4 for carbons C14 and C15, respectively. The signal for C14 was shifted to the lower field because it is a quaternary carbon at the ring junction of rings C and D. Taraxerone was found in leaves of *Elaeophorbium drupifera* Ahiahonu Pearson, (2007). and *Embelia schimperi* leaves Manguro (2006).

Compound 3:

was obtained as Colourless needles with m.p. 139-140°C. The mass spectrum (Ms) exhibited a molecular ion peak at m/z 426 which consistent with the formula molecule $C_{30}H_{50}O$. 1H NMR spectrum showed eight singlet signals representing eight methyl groups at δ 0.81, 0.83, 0.91, 0.93, 0.96, 0.98 and 1.09 for hydrogen H25, H28, H26/H30, H24, H29, H23 and H27 respectively. Double doublet signals appeared at δ 5.53 with coupling constant of 5.5 and 2.6 Hz for the olefinic proton H15 coupled with protons of H16. The significant signal for H3 appeared at δ 3.20 as a double doublet. APT NMR spectrums displayed 30 carbons, of which there were eight methyls, nine methylenes, five methines and eight quaternary carbons. The double bond in compound 3 was represented by two signals at δ 158.3 and 117.1 for carbons C14 and C15 respectively. The signal for the carbon C3 which carries the hydroxyl group appeared at δ 79.3.

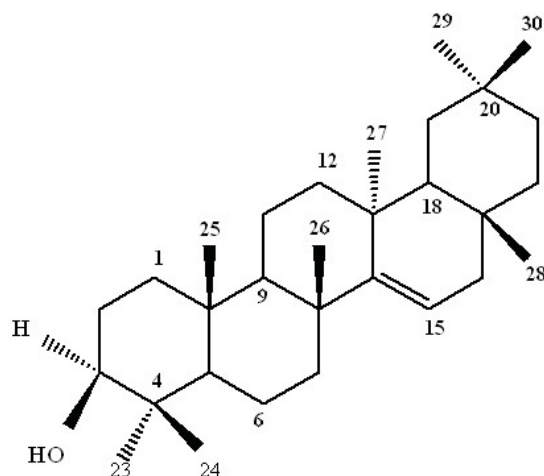


Fig. 3

Taraxerol was found in stem of *Opuntia dillenii* Jiang Jianqin *et al.*, (2006) and *Euphorbia* Jassbi Amir Reza, 2006.

Conclusion:

The isolation and identification of stigmasterol (1), taraxerone (2) and taraxerol (3) from the root bark of *Phyllanthus columnaris* was the first ever to be done and reported from this plant. The work was carried out by means of various physical (solvent extraction, radial chromatography) and spectral techniques.

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REFERENCES

- Agharkar, S.P., 1991. Medicinal Plants of Bombay Presidency, Scientific Publ., Jodhpur, India, 120–122.
- Whitmore, T.C., 1972. Tree Flora of Malaya – A Manual for Foresters Vol 1&2. Longman, Malaysia.
- Kirtikar K.R. and Basu B.D., 1935. Indian Medicinal Plants, III, pp: 2225.
- Ganapaty, S., S.T. Pannakal, G.V.K.I. Srilakshmi, P. Lakshmi P.G. Waterman and R. Brun, 2008. Pumilanol, an antiprotozoal isoflavanol from *Tephrosia pumila*. *Phytochemistry Letters*, 1(4): 175-178.
- Iranshahi Mehrdad, Hosseini Seyyed Tahmineh, Shahverdi Ahmad Reza, Molazade Kamyar, Khan Saleha Suleman and Ahmad Viqar Uddin 2008. Diversolides A..G, guaianolides from the roots of *Ferula diversivittata*. *Phytochemistry*, 69(15): 2753-2757.
- Misra Laxminarain, Mishra Priyanka, Pandey Archana, Rajender S. Sangwan, SangwanNeelam S. and Tuli Rakesh 2008. Withanolides from *Withania somnifera* roots. *Phytochemistry*, 69(4): 1000-1004.
- Ahiahonu Pearson and Goodenowe Dayan B.2007. Triterpenoids from leaves of *Elaeophorbium drupifera*. *Fitoterapia*, 78(5): 337-341.
- Manguro Lawrence Onyango Arot, Okwiri Steve Onyango and Lemmen Peter, 2006. Oleanane-type triterpenes of *Embelia schimperi* leaves. *Phytochemistry*, 67(24) 2641-2650.
- Jiang Jianqin, Li Yanfang, Chen Zhen, Min Zhida and Lou Fengchang, 2006. Two novel C₂₉-5 β -sterols from the stems of *Opuntia dillenii*. *Steroids*, 71(13-14): 1073-1077.
- Jassbi Amir Reza, 2006. Chemistry and biological activity of secondary metabolites in *Euphorbia* from Iran. *Phytochemistry*, 67(18): 1977-1984.