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Phytochemical Studies of *Phyllanthus debilis*

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Abstract – Two Lignans Phyllanthin and Hypophyllanthin, and a steroid β-sitosterol has been isolated from the leaves of *Phyllanthus debilis* and their structures were established by spectral analysis and direct comparison with authentic samples. This is the first report of occurrence of these compound from *P.debilis.*
Key words – *Phyllanthus debilis,* Euphorbiaceae, Lignans, Steroid.

Introduction

Phyllanthus amarus and *Phyllanthus debilis* (Indira Balachandran and Sivarajan 1994) are closely related and similar looking species commonly available in India. *P. amarus* is widly distributed throughout India, while *P. debilis* has its distribution restricted towards Southern India. These are given in stimulating sluggish liver and as a tonic. Isolation of a bitter lignan, phyllanthin and a nonbitter lignan, hypophyllanthin from *P.niruri* was reported earlier (Row *et al.*, 1970). Isolation of phyllanthin and hypophyllanthin from *P.amarus* was also reported (Aimon *et al.,* 1993). *P. fraternus* in native probably to west Pakistan and western India and has been introduced into Africa and West indies .Its closest relative is *P.debilis.* The two species though appearing distinct, are considered to be two allopatric sub-species of a single species, which interfered when they come together (The Wealth of India, 1950). *P.debilis* has been proved to be a better hepatoprotectant than *P.amarus* at dose of 0.66 g/kg against CCl_4 (0.7 ml/kg) induced liver dysfunction (Sane *et al.,* 1995).The efficacy of the aqueous extract of leaves was compared with roots and stems of *P.debilis* against carbontetrachloride induced rat liver dysfunction (Shah *et al.,* 2002). The potential hepatoprotective action of the extract of *P. debilis* whole plant in various solvents on carbontetrachloride induced liver damage rat model was investigated (Shah *et al.,* 1999). However, no chemical work appears to have been done on this plant. The paper work deals with the isolation of Chemical constituents from *P.debilis.*

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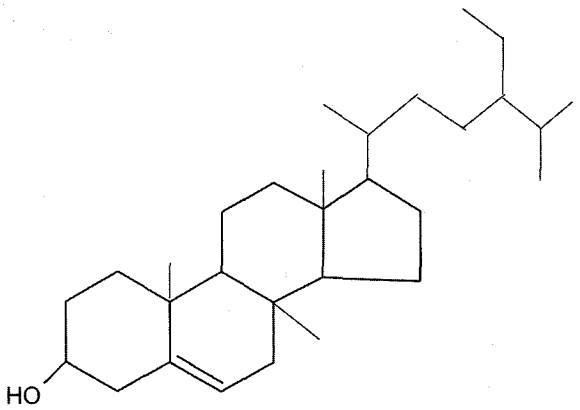
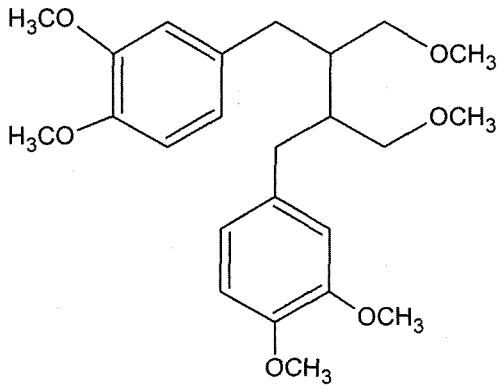
Experimental

The whole plant of *P.debilis* was collected personally from Udupi district and identified by Dr. K Gopal Krishna Bhat, Taxonomist, Department of Botany, Poornaprajna College, Udupi. The Specimen Sample is kept in the department. Whole plant of *P.debilis* were washed with water and sundried. They were crushed into fine powder. The dried powder (3 kg) was mixed with lime (150 g) and water. Then shaken with Petroleum ether (60°C – 80°C) and the petroleum ether extract was concentrated to remove the solvent. The bright yellow coloured residue (45 g) was dissolved in 500 ml of ethyl alcohol, boiled for 15 min. on a water bath, cooled in a refrigerator for 30 minutes. Precipitation of wax took place, it was then filtered and the filtrate was concentrated. The concentrate (40 g) was subjected to column chromatography. Size of the column is 600 mm long and 30 mm in diameter. The concentrate was loaded into a silica gel column (150 g) prepared in petroleum ether. The column was then eluted with petroleum ether, benzene, chloroform and ethyl acetate in a polarity gradient manner. Totally 450 fractions of 25 ml each were collected. Fractions 171 – 178 eluent gave 2 spots on TLC. These were combined and concentrated to about 10 ml when it yielded a white solid. It was separated by preparative TLC using silica gel (petroleum ether: benzene; 8:2) into compound 1 and 2. Pure compound **1**(58.2 mg) was obtained through recrystallization from petroleum ether. Pure compound **2** (49.4 mg) was obtained through recrystallization from petroleum ether. Fraction 180 – 188 gave 1 spot on TLC. These were combined and concentrated (compound **3**). Pure compound **3** (52.3 mg) was obtained through recrystallization.

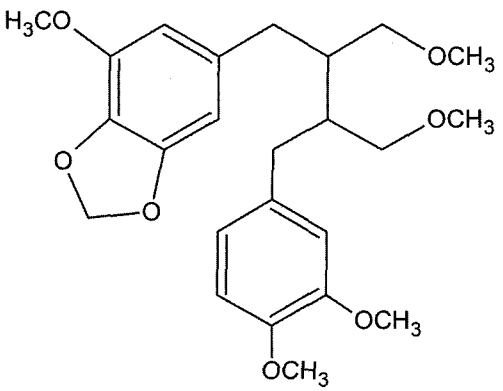
Compound 1 (β-sitosterol) – compound **1**, crystallized from $CHCl_3$ as white crystals, m.p. 138°C should positive salkowskis test and Liebermann Burchards test for steroids. IR(KBr cm^{-1}) γ_{max} 3417 (OH), 2942, 2800 (C-H stretching in CH_2 and CH_3), 1628 (C=C stretching), 1461 (C-H deformation in CH_3), 1377 (C-H deformation in gemdimethyl), 1056 (C-O stretching of secondary alcohol). Its molecular formula was found to be $C_{29}H_{50}O$ (M^+414). The 396, 355, 329, 303 273,255, 213,178, 145, 95, 57. ^{1}H-NMR (300 MHz. $CDCl_3$) δ: 5.36 (d, *J*=1.4 Hz), 3.5 (dd, *J*=2.8 Hz). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 145 (C-5), 121 (C-6), 56 (C-17), 50 (C-20), 45 (C-13), 40 (C-14), 36 (C-22), 37 (C-15), 33 (C-1), 35 (C-8), 34 (C-23), 35.1 (C-10), 32 (C-16), 33 (C-7), δ 29.12 (C-25), 30.25 (C-24), 30 (C-14), 28.24 (C-27), 28 (C-29), 31.6 (C-26), 18.7 (C-28). It was found to be identifical with β-sitosterol on comparison with authentic sample (mixed. m.p, CO-TLC, and Superimposable IR).



Compound 2 (Phyllanthin) – compound **2**, crystallized from $CHCl_3$ as white needles, m.p: 96°C showed bluish green colour with $MeOH/H_2SO_4$ (9:1), IR (KBrcm^{-1}) γ_{max} 1517, 1482, 1463, 1446, 1419, 1313, 1269, 1247, 1236, 1178, 1159, 1139, 1109, 1055, 1041, 1026, 964, 952, 858, 817, 788, 767 and 752. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.59 (1H, d, *J*=1.8 Hz, 2 and 2^1 H), 6.57 (1H, d, *J*=8.1, 5 and 5^1-H), 6.63 (1H, dd, *J*=1.8 Hz and 8.1 Hz, 6 and 6^1-H), 2.62 (2H, dd, *J*=13.8 Hz and 7.3 Hz, 7-H), 2.66 (2H, dd, *J*=13.8 Hz and 7.5 Hz, 7-H), 2.03 (1H, m, H-8), 3.26 (2H, dd, *J*=13.8 Hz and 7.8 Hz, H-9), 3.3 (2H, dd, *J*= 13.8 Hz and 5.4, H-9^1), 3.8 (3H, s, H-3 and 3^1-OCH_3). ^{13}C-NMR (75 MHz, $CDCl_3$), δ : 133 (C-1 and 1^1), 112 (C-2 and 2^1), 148 (C-3 and 3^1), 147 (C-4 and 4^1), 111 (C-5 and 5^1), 120 (C-6 and 6^1), 34 (C-7 and 7^1), 40 (C-8 and 8^1), 72 (C-9 and 9^1), 56 (C-3 and 3^1-OCH_3), 55 (C-4 and 4^1-OCH_3), 58 (C-9 and 9^1-OCH_3). It was found to be identical with Phyllanthin on comparison with authentic sample (mixed.m.p, TLC and Superimposable IR).



Compound 3 (Hypophyllanthin) – compound **3**, crystallized from $CHCl_3$ as white needles, m.p:128°C, showed violet colour with $MeOH/H_2SO_4$ (9:1). Unlike Phyllanthin, it yields a permanent emerald green colour in Labat test indicating the Presence of a methylene dioxy group in the molecule. IR (KBrcm^{-1}) γ_{max} 1593, 1515, 1506, 1463, 1425, 1369, 1325, 1259, 1228, 1201, 1151, 1126, 1103, 1043, 1072, 1026, 964, 939, 920, 826, 823, 808, 794, 754, 693 and 638. ^{1}HNMR (300 MHz $CDCl_3$) δ: 4.0 (1H, d, *J*=7.8 Hz, H-1), 1.9 (1H, m, H-2), 3.24 (2H, dd, H-2a), 1.98 (1H, m, H-3), 3.6 (2H, dd, *J*=9.6 Hz and 6.2 Hz, H-3a), 2.75 (2H, dd, *J*=15.9 Hz, H-5), 6.62 (1H, *J*=2.0 Hz, H-2^1), 6.75 (1H, d, *J*=8.0 Hz, H-5^1), 6.6 (1H, dd, *J*=8.0 Hz and 2.0 Hz, H-6^1), 3.32 (3H, s, H-2a-OCH_3), 3.85 (3H, s, H-4-OCH_3), 5.65 (2H, d, *J*=1.4 Hz, -OCH_2O). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 42 (C-1), 46 (C-2), 72 (C-2a), 37 (C-3), 76 (C-3a), 33 (C-4),, 132 (C-4a), 107 (C-5), 142 (C-6), 134 (C-7), 147 (C-8), 115 (C-8a), 138 (C-1^1), 112 (C-2^1), 149 (C-3^1), 147 (C-4^1), 111 (C-5^1), 121 (C-6^1) 59 (C-2a-OCH_3), 59 (C-3a-OCH_3), 57 (C-6-OCH_3), 56 (C-3^1-OCH_3), 56 (C-4^1-OCH_3), 101 (C-OCH_2O-). It was found to be identical with Hypophyllanthin on comparison with authentic sample (mixed m.p., Co-TLC and Superimposable IR).



Results and Discussion

Chromatographic resolution of the petroleum ether extract of the whole plant of *Phyllanthus debilis* furnished compounds A, B, and C which characterized as β-sitosterol, phyllanthin and hypophyllanthin by a detailed spectral analysis i.e., IR, H-NMR and ^{13}C-NMR and direct comparison with authentic samples (mixed m.p CO-TLC and Syperimposable IR). All the above compounds are the first report from this plant.

Acknowledgement

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