Vol. 16 (2013) 44-48

Phytosterols from *Dombeya torrida* (J. F. Gmel.)

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Dombeya torrida **collected from Kinale forest in Kiambu County, Kenya, was Soxhlet extracted with chloroform and by percolation using a dichloromethane:methanol mixture. The extracts were fractionated using normal phase silica gel in an open column. Five compounds were isolated namely friedelin, friedelan-3β-ol, β-sitosterol, taraxerol and stigmasterol. This is the first report of isolation of these compounds from Dombeya torrida. The isolated compounds were identified by means of ^{1}H-NMR, ^{13}C-NMR, DEPT, MS and IR analyses.**

Key words: *Dombeya torrida***,** friedelin, friedelan-3β-ol, β-sitosterol, stigmasterol, taraxerol.

INTRODUCTION

Dombeya torrida (J.F. Gmel.) is a shrub (or tree) with a height range of 6-24 m. Its leaves are broadly ovate, base deeply cordate, apex acuminate, margin serrate to entire, 4-25 by 3-15 cm and densely pubescent. Branches are pubescent when young and glabrous when older. Flowers are white, red at the base inside, in umbels with branched stalks. Its petals are 11-21 mm long while its fruits are round and hairy [1, 2]. The plant grows within an altitude of 1850 to 2700 m [2]. It is widely distributed from Eritrea and Ethiopia southward through Central and East Africa to southern Malawi [3]. In Kenya, *D. torrida* is found in highland forests throughout the country and is splendid when in flower [1].

A decoction of the flowers and bark is taken for indigestion by the Maasai in Kenya and Tanzania, for the treatment of chest pains and colds [3,4,5]. Previous work on *D. torrida* by Chepkwony [6] yielded 3β-hydroxyglutin-5-ene, mansonone E and mansonone F. The objective of this study was to carry out further phytochemical investigation of *Dombeya torrida*.

MATERIALS AND METHODS

Plant material

The *D. torrida* stem-bark, leaves and flowers were collected on 3rd August 2006 and on 17th August 2007 at Kinale forest in Kiambu County, Kenya. The plant specimens were identified at th Department of Botany, University of Nairobi. A voucher specimen (number 2006/002) is deposited at the School of Biological Sciences herbarium, University of Nairobi.

Reagents and solvents

Normal phase thin layer chromatography (TLC) pre-coated plates with silica gel $60F_{254}$ were from Sigma-Aldrich Chemie (Steinheim, Germany). Column chromatography was carried out on silica gel for column chromatography (0.032-0.63 mm) from Sigma-Aldrich Laborchemikalien (Seelze, Germany). Methanol, dichloromethane, n-hexane and chloroform were obtained from Kobian Kenya Ltd (Nairobi, Kenya), while ethyl acetate was from Synerchemie Chemicals (Nairobi, Kenya). All solvents were of general purpose grade and

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were distilled prior to use. Vanillin was acquired from Laboratory Chemicals (Nairobi, Kenya). Concentrated sulphuric acid was from Kanha Laboratory Supplies (Nairobi, Kenya).

Equipment

Extractions were done using a 2,000 ml Soxhlet apparatus (Quickfit, Birmingham, U.K.) and by percolation using a 1 m long glass column of 80 mm internal diameter. The extracts were reduced on a rotary vacuum evaporator (Heidolph VV2000, Heidelberg, Germany) connected to a rotary vane pump (KNF Laboport Neuberger, Freiburg, Germany). Fractions were collected using a Superfrac fraction collector from Pharmacia LKB Biotechnology, Uppsala, Sweden. A Min UV/Vis box (Desaga GmbH, Heidelberg, Germany) was used for visualising developed TLC plates. High resolution mass spectrometric (MS) analysis was done using a GC Mate II Jeol (Tokyo, Japan) mass spectrometer. A Shimadzu Fourier Transform IR Prestige-21 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) was used for infrared (IR) analysis. Nuclear magnetic resonance (NMR) spectroscopic analysis of isolated compounds was carried out using a Mercury Varian 200 MHz NMR spectrometer from Varian Inc. (Palo Alto, California, U.S.A.).

Solvent extraction

The stem-bark, leaves and flowers were air-dried at room temperature, finely ground and stored in closed plastic containers at room temperature until use. About 1 kg of the stem bark powder was extracted with chloroform for 48 h using a Soxhlet apparatus while another 2 kg of the leaf powder was percolated in dichloromethane:methanol (50:50) in an open column. The extracts were filtered and reduced to dryness *in vacuo*.

Column chromatography

The extracts were fractionated using an open column packed with normal phase silica gel for column chromatography (0.032-0.63 mm). The chloroform extract was isocratically eluted using chloroform while gradient elution was carried out for dichloromethane:methanol extract using solvents of increasing polarity starting with hexane, dichloromethane and ethylacetate. Fractions were monitored using thin layer chromatography (TLC) pre-coated plates and those with similar profiles pooled. The pooled fractions were reduced to dryness *in vacuo* and crystallized in chloroform or ethylacetate at room temperature.

Spectroscopic analysis

The isolated compounds were subjected to high resolution mass spectrometric analysis at the University of Cape Town. Infra-red (IR) analysis was done at the Department of Pharmaceutical Chemistry, University of Nairobi. One dimensional NMR analysis (^{1}H-NMR and ^{13}C-NMR) and distortionless enhancement by polarization transfer (DEPT) was carried out at the Department of Chemistry, University of Nairobi.

RESULTS

Friedelin, friedelan-3β-ol, β-sitosterol and stigmasterol were isolated from the *D. torrida* chloroform extract. The *Dombeya torrida* dichloromethane:methanol extract availed friedelin, β-sitosterol, stigmasterol and taraxerol. The spectral data of the 5 compounds namely friedelin, friedelan-3β-ol, β-sitosterol, stigmasterol and taraxerol were in agreement with those reported in literature [7-12]. The chemical structures of the isolated compounds is shown in Figure 1.

Friedelin: White star-like crystals from ethyl acetate; m.p. 261-265 ^{o}C; IR ν_{max} (KBr) cm^{-1}: 2962.66-2868.15, 1710.86, 1460.11; MS *m/z* (relative intensity %): 426.47 (M^+, 27), 425.46 (M^+-H, 75), 411.53 (M^+-CH_3, 5), 410.54 (15), 341.58 (3), 302.55 (15), 301.58 (37), 273.57 (34), 272.61 (54), 247.68 (21), 231.61 (34), 230.61 (29), 217.69 (48), 204.70 (56), 178.74 (45), 162.76 (46), 148.77 (29), 136.80 (46), 124.82 (87), 122.81 (85), 108.82 (89), 94.83 (100), 68.86 (96), 66.86 (40), 54.87 (57), 41.17 (29); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.70, 0.88, 0.89, 1.02, 1.07, 1.20, 1.4, 1.6, 2.3; ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 22.51 (C-1), 41.51 (C-2),

213.51 (C-3), 58.44 (C-4), 42.37 (C-5), 41.76 (C-6), 18.46 (C-7), 53.32 (C-8), 37.66 (C-9), 59.68 (C-10), 35.56 (C-11), 30.73 (C-12), 39.91 (C-13), 38.51 (C-14), 32.34 (C-15), 36.23 (C-16), 30.22 (C-17), 43.00 (C-18), 35.25 (C-19), 28.40 (C-20), 32.64 (C-21), 39.47 (C-22), 7.06 (C-23), 14.88 (C-24), 18.18 (C-25), 20.49 (C-26), 18.90 (C-27), 32.01 (C-28), 35.84 (C-29), 32.99 (C-30).

Fridelan-3β-ol: White star-like crystals from ethyl acetate; IR ν_{max} (KBr) cm^{-1}: 3624.25, 3479.46, 2937.59, 2866.22, 1462.04, 1382.96; MS *m/z* (relative intensity %): 428.39 (M^+, 82), 426.38 (13), 424.37 (4), 413.38 (M^+-CH_3, 54), 395.39 (9), 346.33 (5), 341.32 (4), 276.25 (24), 275.23 (69), 273.24 (8), 275.23 (69), 261.26 (15), 259.24 (18), 257.23 (16), 248.22 (23), 234.20 (34), 233.19 (38), 231.20 (42), 220.18 (41), 205.20 (46), 177.16 (48), 165.13 (100), 149.12 (28), 137.13 (35), 125.13 (64), 123.12 (57), 121.10 (51), 96.10 (91), 95.09 (91), 81.07 (53), 69.07 (66); ^{1}H-NMR (400 MHz, $CDCl_3$ + Acetone-D_6) δ: 0.16 0.19, 0.23, 0.27, 0.29, 0.31, 0.46, 0.63, 0.66, 0.81, 0.84, 0.87, 0.99, 1.06, 1.14, 1.19, 1.36, 1.38, 1.39, 2.10, 3.00, 7.00; ^{13}C-NMR (50 MHz, $CDCl_3$ + Acetone-D_6) δ: 16.05 (C-1), 35.01 (C-2), 71.46 (C-3), 49.16 (C-4), 38.12 (C-5), 41.63 (C-6), 17.33 (C-7), 52.96 (C-8), 36.86 (C-9), 61.28 (C-10), 35.32 (C-11), 30.54 (C-12), 37.69 (C-13), 39.39 (C-14), 32.02 (C-15), 35.79 (C-16), 30.40 (C-17), 42.58 (C-18), 35.32 (C-19), 27.82 (C-20), 32.51 (C-21), 38.96 (C-22), 11.37 (C-23), 15.67 (C-24), 17.92 (C-25), 19.75 (C-26), 18.29 (C-27), 31.68 (C-28), 34.56 (C-29), 31.38 (C-30).

Beta-sitosterol and Stigmasterol mixture: Beta-sitosterol and Stigmasterol were isolated together as colourless needle-like crystals from ethyl acetate. Melting point: 136-145 °C. Literature has many examples of these compounds isolated together [8].

Beta-sitosterol: ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 37.48 (C-1), 31.86 (C-2), 72.03 (C-3), 42.51 (C-4), 140.97 (C-5), 121.95 (C-6), 32.13 (C-7), 32.13 (C-8), 50.35 (C-9), 36.73 (C-10), 21.31 (C-11), 39.99 (C-12), 42.51 (C-13), 56.99 (C-14), 24.53 (C-15), 28.48 (C-16), 56.17 (C-17), 12.09 (C-18), 19.63 (C-19), 36.38 (C-20), 19.01 (C-21), 34.16 (C-22), 26.27 (C-23), 46.04 (C-24), 29.36 (C-25), 20.06 (C-26), 19.26 (C-27), 23.28 (C-28), 12.21 (C-29).

Stigmasterol: ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 37.48 (C-1), 31.86 (C-2), 72.03 (C-3), 42.51 (C-4), 140.97 (C-5), 121.95 (C-6), 32.13 (C-7), 32.13 (C-8), 50.35 (C-9), 36.73 (C-10), 21.31 (C-11), 39.91 (C-12), 42.51 (C-13), 57.09 (C-14), 24.53 (C-15), 29.15 (C-16), 56.17 (C-17), 12.09 (C-18), 19.26 (C-19), 40.74 (C-20), 21.45 (C-21), 138.56 (C-22), 129.49 (C-23), 51.47 (C-24), 32.13 (C-25), 19.63 (C-26), 20.06 (C-27), 25.64 (C-28), 12.49 (C-29).

Taraxerol: Colourless sugar like crystals from dichloromethane; m.p. 281-284 °C; IR ν_{max} (KBr) cm^{-1}: 3487.30, 2964.59-2860.43, 1641.42, 1467.83, 1454.33, 1375.25, 1031.92 and 999.13; MS m/z (rel. int. %): 428.31 (M^++2, 3), 426.30 (M^+, 48), 411.27 (17), 303.20 (14), 302.20 (57), 287.17 (30), 269.17 (13), 231.16 (7), 218.15 (26), 205.15 (29), 204.14 (100), 191.14 (10), 189.12 (16), 135.08 (26), 81.05 (11), 69.05 (15); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.80 (s), 0.82 (s), 0.90 (s), 0.92, 0.94, 0.97, 1.08, 1.25, 1.32, 1.58, 1.96 (m, 2H), 3.18 (m , 1H), 5.53 (dd, 1H); ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 37.93 (C-1), 27.36 (C-2), 79.29 (C-3), 39.18 (C-4), 55.73 (C-5), 19.01 (C-6), 35.32 (C-7), 38.98 (C-8), 48.93 (C-9), 37.77 (C-10), 17.72 (C-11), 36.01 (C-12), 37.93 (C-13), 158.29 (C-14), 117.09 (C-15), 36.89 (C-16), 38.21 (C-17), 49.49 (C-18), 41.52 (C-19), 29.02 (C-20), 33.90 (C-21), 33.30 (C-22), 28.21 (C-23), 15.67 (C-24), 15.67 (C-25), 30.04 (C-26), 26.13 (C-27), 30.15 (C-28), 33.57 (C-29), 21.54 (C-30).

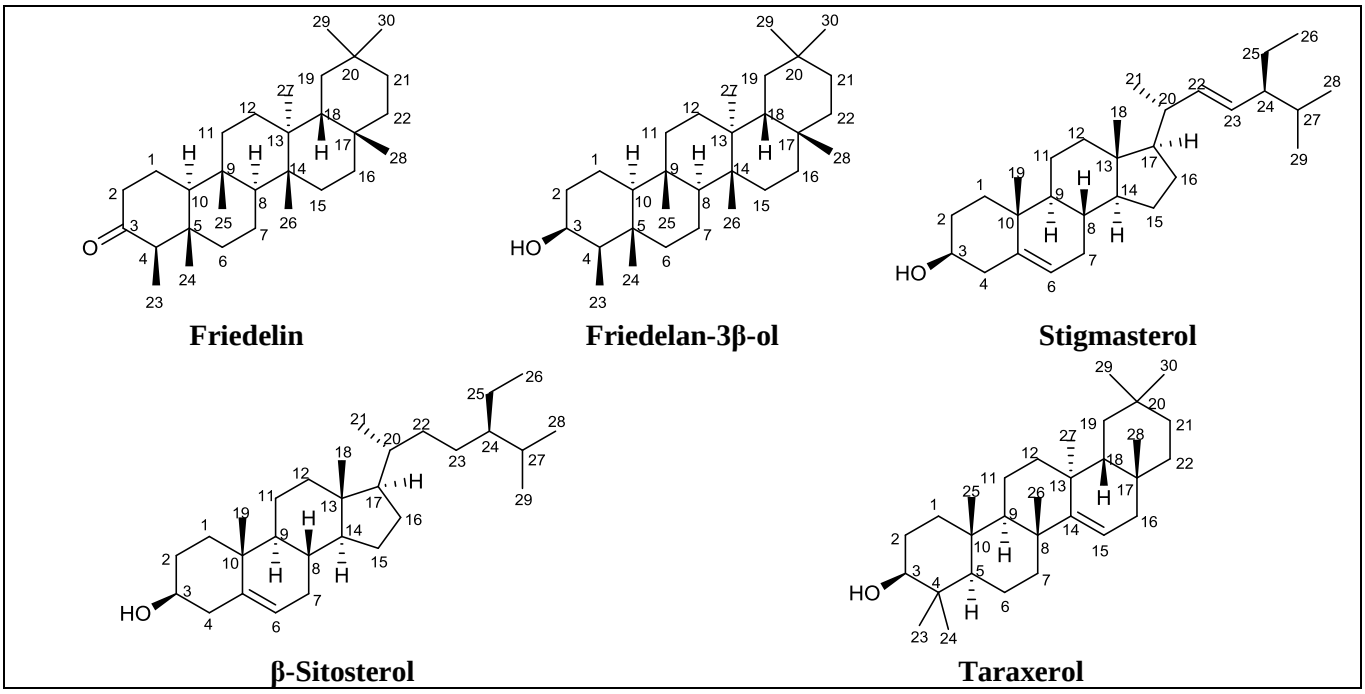

Figure 1. Compounds isolated from *Dombeya torrida*

CONCLUSION

The study provides new knowledge regarding the phytochemistry of *D. torrida.* Five compounds were isolated from *D. torrida* and identified as friedelin, friedelan-3β-ol, β-sitosterol, stigmasterol and taraxerol. This is the first report of the isolation of these compounds from *D. torrida*.

ACKNOWLEDGEMENTS

The authors are grateful to *Deutsch Academischer Auslader-Dienst* (DAAD) for funding the study and Prof. Abiy Yenesew of University of Nairobi and Prof. Kelly Chibale of University of Cape Town for assisting in MS and NMR analysis. Gratitude also goes to Mr. Simon Mathenge who assisted in collection and identification of the plant.

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