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ORIGINAL ARTICLE

Neoraudiol, a new isoflavonoid and other antimicrobial constituents from the tuberous root of *Neorautanenia mitis* (A. Rich) Verdcourt

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Abstract A bioassay-directed fractionation of *n*-hexane and chloroform extracts of the tuberous roots of *Neorautanenia mitis* resulted in the isolation of a new isoflavonoid, named neoraudiol (**1**) and five known compounds: rautandiol A (**2**), neoduline (**3**), neotenone (**4**), pachyrrhizine (**5**) and 12a-hydroxylrotenone (**6**). The structure of the new compound was established on the basis of spectroscopic studies and comparison with known compounds. Isolated compounds exhibited broad spectrum antimicrobial activity ranging from 15 to 80 mg/ml, the most significant activity (MIC15.10 ± 0.5 mg/ml) was exhibited by neoraudiol. Structure–activity relationship is described.
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1. Introduction

Neorautanenia mitis (A. Rich) Verdcourt (Fabaceae) is a leguminous subshrubby plant found growing in rocky soil. *N. mitis* is native to the Central, South and West Africa and one of the 10 species of *Neorautanenia* (Burkill, 1995). Previously reported constituents of *N. mitis* include isoflavones, coumarin derivatives and pterocarpans (Cromble and Whiting, 1962; Luc et al., 1987; Joseph et al., 2004; Yojiro et al., 2006). The root decoction of *N. mitis* is reported in traditional medicine as fish poison and for killing bilharzias-carrying fresh water snails; powdered root of *N. mitis* is reputed for its insecticidal and insect-repellant properties, treatment of syphilis, female frigidity and skin infections like scabies and rashes (Luc et al., 1987; Heydoricks et al., 1992; Leticia et al., 2008). The root petroleum extracts of *N. mitis* have significant acaricidal activity against female ticks, larvacidal and mosquitocidal activities against larvae of *Anopheles gambiae* and *Culex quinquefaciatus* mosquitoes (Luc et al., 1987; Joseph et al., 2004). Cytotoxic activities of the EtOAc extract of *N. mitis* in human MCF-7 breast and A-549 lung cancer have been reported (Yojiro et al., 2006). Aqueous extract of *N. mitis* is reported to have dose-dependent anti-ioceptic activity at 12.5 and 50.0 mg/ml p.o. in mice and slight inflammatory activity at 25 and 50 mg/ml p.o. (Vongtau et al., 2005). Neotenone, pachyrrhizine, neorautanone, neoduline, nepseudin, 12a-hydroxyl rotenone, rautandiol A and B are implicated for these activities. However, a study on the antimicrobial activity of constituents from *N. mitis* is rare in the literature. Flavonoids and pterocarpans are reported as important phytoalexins (Cecile et al., 2003; Leticia et al., 2008). In this context, our continuing interest in antimicrobial agents of potential medicinal use led

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us to a study of *N. mitis*, a plant widely used in indigenous medical practice in Nigeria for the treatment of scabies and rashes. Herein, the isolation and structural elucidation of a new isoflavonoid, neoraudiol (**1**), along with five known compounds [rautandiol A (**2**), neoduline (**3**), pachyrrhizine (**4**), neotenone **(5)** and 12a-hdroxyrotenone **(6)**] from the tuberous roots of *N. mitis* are described. The chemical structures of compound **1** were established by detailed 1D and 2D NMR, EIMS, UV, IR spectroscopic data, and comparison of those data with published values. Compounds **1**–**6** were evaluated *in vitro* for their growth inhibition on three Gram-negative bacteria [(*Eschericia coli*, ATCC 1053, *Pseudomonas auerginosa*, ATCC 278660), *Salmonella typhii* (Lab. Stock)], two Gram-positive bacterial strains (*Bacillus substilis*, ATCC 6052, *Staphylococcus aureus*, ATCC 13705) and two fungi: *Candida albicans* and *Aspergillus niger*.

2. Materials and methods

2.1. General procedures

Melting points (mp) uncorrected were determined with the aid of Gallenkamp (Phillip Harris, England) melting apparatus. Analytical and preparative chromatographic procedures utilised adsorption chromatography. Analytical thin layer Chromatography (TLC) was performed using commercial pre-coated plates (Kieselgel, $60PF_{254}$, 0.25 mm thick on polyester backing (Merck, Damstadt, Germany), spots were visualised in iodine vapour. Column chromatography was performed on open column silica gel (Merck, 60, 70–230 mesh). IR spectra were recorded on Unican FT-IR 2000 model spectrophotometer, in Nujol and KBr pellets. UV spectrum was recorded on a Unikon model 930 and Lambda 3B model spectrophotometers. EIMS (70 eV) and GC–MS were acquired using JEOL MS Revile (Direct probe). ^{1}H- and ^{13}C-NMR spectra were acquired on a Bruker WP 120054 (400 MHz, 500 MHz for ^{1}H- and 100 MHz for ^{13}C-NMR) spectrometer with TMS as internal reference in $CDCl_3$ and CD_3OD. Chemical shift values are expressed in ppm and coupling constant (*J*) in Hertz (Hz). *Gentamicin* was used as reference antimicrobial drugs while *thioconazole* served as reference antifungal drug.

2.2. Plant material

The tuberous roots of *N. mitis* were collected from the rocky soil along Suleja-Abuja road, Niger State, Nigeria, in June 2007. The plant was botanically identified by Mr. O.A. Ohaeri of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu Abuja, Nigeria. Voucher specimen was deposited in the herbarium of NIPRD with accession number NIPRD 11225.

2.3. Extraction and isolation

Dried and powdered tuberous roots of *N. mitis* (1.5 kg) were exhaustively and successively extracted by percolation with *n*-hexane and $CHCl_3$ (72 h each, reflux). The extract was concentrated to yield 28 g hexane and 35 g $CHCl_3$ extracts.

The $CHCl_3$ extracts of *N. mitis* (6 g) were chromatographed on silica gel packed column, eluent: *n*-hexane–Et_2O gradient, 100 ml. Fractions (Frs.) 1–42 (*n*-hexane), 43–69 (hexane–Et_2O, 9:1), 70–96 (hexane–Et_2O, 8:2), 97–143 (hexane–Et_2O, 7:3), 144–166, (hexane–Et_2O, 2:3) and 167–186 (Et_2O). Identical fractions were grouped into six subfractions on the basis of TLC. Frs 1–42 (A), 43–69 (B), 72–110 (C), 111–143 (D), 144–169 (E) and167–186 (F). Fr. E (100 mg) was rechromatographed on a silica gel narrow column (hexane–Et_2O stepwise gradient, 50 ml), eluting progressively with 100% *n*-hexane up to 50% hexane–Et_2O, yielding neoraudiol (**1**) and rautandiol (**2**).

The *n*-hexane extract of *N. mitis* (5 g) was chromatographed on a silica gel packed column, eluting with hexane–Et_2O stepwise gradient. Fractions obtained were bulked into four (A–D) on the basis of TLC. Subfractions A and C yielded compounds **3** and **4**. Fraction D (400 mg) was rechromatographed in a silica gel narrow column, eluted gradually in hexane–Et_2O gradient. Subfractions 25–35, eluted with 70% hexane in Et_2O afforded yellow solid, pachyrrhizine (65 mg, **5**). The subfractions 45–64 eluted with 60% hexane in Et_2O afforded yellow powder, 12a-hydroxy rotenone (500 mg, **6**) after recrystallisation in hexane–EtOAc (8:1, vol/vol).

2.3.1. Compound *1* (neoraudiol)

Compound **1** was eluted with 75% hexane–Et_2O and isolated as light-green powder (65 mg), after recrystallisation in hexane–EtOAc (3:1, vol/vol), mp 205–206° C; 1R (υ_{max}, cm^{-1}, KBr): 3781.2, 1665.7, 1593.8, 1475.2 and 1162.3. UV [(λ_{max}, nm) (ε_{max})]: 389.8 (0.04), 323 (0.12) and 233.8 (0.89); ^{1}H-NMR (CD_3OD, 400 MHz) δ (ppm): 8.49 (1H, s, H-5), 8.25 (1H, s, H-2), 7.94 (1H, d, $J = 2$ Hz H-2″), 7.76 (1H, s, H-8), 7.10 (1H, d, $J = 8$ Hz H-5′), 7.05 (1H, dd, $J = 2$, 1.6 Hz H-3″), 6.41 (1H, dd, $J = 8$, 2.0 Hz H-6′), 6.38 (1H, d, $J = 2$ Hz H-2′); ^{13}C-NMR (100 MHz, CD_3OD) δ (ppm): 180 (C-4), 160 (C-8a), 159 (C-7), 157.4 (C-2), 149.7 (C-2″), 133.1 (C-2′), 123.5 (C-3), 122 (C-1′), 120 (C-5), 119.4 (C-4a), 119 (C-6), 108.3 (C-6′), 107.9 (C-4′), 106.1 (C-3″), 104.5 (C-5′), 104.1 (C-3′), and 100.9 (C-8). E1MS [(*m*/*z* (rel. int. (%)]: 293.6 [(M^{+}), 13.34)], 276.7 (11.1), 264.7 (2.4), 236.7 (3.3), 160.8 (100), 146.9 (12.1), 133.9 (33.9), 119.9 (11.3), 104.9 (21.1), 90.9 (44.4), 76.9 (25.8), 65.0 (13.5), 50.0 (13.7).

2.3.2. Compound *2* (rautandiol)

Compound **2** was eluted with 50% hexane–Et_2O and isolated as grey powder (83 mg), upon recrystallisation in hexane–EtOAc (1:1, vol/vol), mp 210–211° C (lit mp 210–211° C); ^{1}H-NMR (CD_3OD, 400 MHz) δ (ppm): 7.17 (1H, s, H-1), 7.06 (1H, d, $J = 8$ Hz, H-7), 6.31(1H, dd, $J = 8$, 3H, s, H-4′), 6.26 (1H, s, H-4), 4.18 (1H, dd, $J = 9.6$, 3.1 Hz, H-6_{eq}), 3.51(1H, dd, $J = 10.5$, 9.6 Hz H-6_{ax}), 3.50–3.46 (1H, m, H-6a), 1.24 (3H, s, H-5′); ^{13}C-NMR (CD_3OD, 100 MHz), δ (ppm): 162.1(C-10a), 159.9 (C-9), 156.6 (C-4a), 155.7 (C-3), 133.3 (C-1), 126.1 (C-7), 119.6 (C-7a), 115.5 (C-2), 114.5 (C-1a), 108.8 (C-8), 105.4 (C-4), 98.9 (C-10), 80.01 (C-11a), 78.6 (C-3′), 70.72 (C-2′), 68.3 (C-6a), 41.2 (C-6), 31.7 (C-1^{1}), 26.1 (C-4′), and 21.1 (C-5′); E1MS (*m*/*z* (rel. int., %): 340.9 [$(M + H)^{+}$, 29.1)], 339.9 (94.5), 338.9 (13.3), 320.9 (2.5), 306.8 (6.8), 268.9 (100), 140.9 (5.2), 198.9 (9.3), 170.9 (4.9), 152.9 (4.1), 138.9 (3.2), 91.0 (9.6), 68.9 (40.7).

2.3.3. Compound *3* (neoduline)

Compound **3** was eluted with 90% hexane–Et_2O and isolated as white powder (95 mg), mp 216–217° C (lit mp 217° C); ^{1}H-NMR: (400 MHz, $CDCl_3$), δ (ppm): 7.76 (1H, s, H-1), 7.57 (1H, d, $J = 2.5$ Hz, H-2″), 7.10 (1H, s, H-4), 6.76 (1H,

s, H-7), 6.45 (1H, s, H-10), 6.73 (1H, dd, J = 2.5, 1 Hz H-3″), 5.94 (1H, d, J = 1.5 Hz) H-4), 5.91 (1H, d, J = 1.5 Hz, H-2‴), 5.68 (1H, d, J = 7 Hz), 4.29 (1H, dd, J = 11, 5 Hz, H-6_{eq}), 3.78 (1H, dd, J = 11, 11 Hz, H-6_{ax}), 3.60 (1H, ddd, J = 11, 7, 5 Hz, H-6a); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 155.7 (C-3), 154.3 (C-10a), 153.5 (C-4a), 145.1 (C-2′), 122.9 (C-1), 122.4 (C-2), 117.9 (C-7a), 116.7 (C-1a), 106.3 (C-3″), 101.3 (C-2‴), 99.9 (C-4), 93.9 (C-10), 79.3 (C-11a) and 40.6 (C-6a); EIMS: m/z [rel. int. (%)]: 307.6 (M^+, 88.3), 306.7 (13.2), 293.7 (20.1), 278.7 (6.6), 264.9 (2.6), 220.8 (3.8), 184.9 (5.5), 170.8 (10.4), 57.1 (100), 152.8 (3.3), 138.8 (3.9), 120.98 (3.1), 106.96 (6.4), 90.9 (59.1), 76.9 (12.7).

2.3.4. Compound *4* (neotenone)

Compound **4** was eluted with 80% hexane–Et_2O as greenish-white solid (100 mg), after recrystallisation in hexane–EtOAc (3:1), mp 157–158° C (lit mp 156° C); ^{1}H-NMR [($CDCl_3$), 500 MHz), δ (ppm)]: 8.37 (1H, s, H-5), 7.59 (1H, d J = 2 Hz, H-2″), 7.08 (1H, s, H-8), 6.78 (1H, dd J = 2, 1 Hz H-3″), 6.63 (1H, s, H-6′), 6.58 (1H, s, H-3′), 5.92 (1H, s, H-2‴), 4.59 (1H, dd J = 11.2, 11.6 Hz H-2), 4.51 (1H, dd J = 11.2, 5.6 Hz, H-2), 4.31(1H, dd J = 11.6, 5.6) and 3.71 (3H, s, OCH_3); ^{13}C-NMR [(100 MHz, $CDCl_3$), δ (ppm)]: 193.0 (C-4), 159.9 (C-8a), 159.6 (C-7), 153.1 (C-2′), 148.0 (C-4′), 146.0 (C-2″), 141.7 (C-5′), 123.0 (C-1′), 121.0 (C-5), 118.8 (C-4a), 115.8 (C-6), 109.0 (C-6′), 107.0 (C-3″), 101.3 (C-2‴), 99.8 (C-8), 95.5 (C-3′), 71.5 (C-2), 56.5 (–OCH_3) and 48.0 (C-3).

2.3.5. Compound *5* (pachrrhizine)

Compound **5** was eluted from column with 7:3 hexane–Et_2O and recrystallised from hexane/EtOAc solvent mixture (7:3, vol/vol) to afford a yellow solid [65 mg], mp 206–207° C (lit mp 204–205° C); ^{1}H-NMR [($CDCl_3$, 400 MHz,), δ (ppm)]: 7.79 (1H, s, H-4), 7.68 (1H, s, H-5), 7.67(1H, d, J = 2 Hz H-2″), 7.49 (1H, s, H-8), 6.89 (1H, s, H-6′), 6.82 (1H, dd J = 2, 1 Hz), 6.63 (1H, s, H-3′), 5.96 (1H, s, H-2‴) and 3.77 (3H, s, br-OCH_3); MS: 336 (M^+, 100%), 321, 305, 293, 265, 249.

2.3.6. Compound *6* (12a-hydroxy rotenone)

Compound **6** was eluted from column with 3:2 hexane–Et_2O as a yellow solid (500 mg, mp 86–87° C (lit mp 87–88° C); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.81 (1H, d, J = 8.6 Hz H-11′), 6.73 (1H, d, J = 8.6 Hz, H-10), 6.56 (1H, s, H-1), 6.49 (1H, s, H-4), 5.27 (1H, m, H- 5′), 4.59 (2H, m, H-6), 4.6–5.1 (2H, m, br H-7′), 4.55 (2H, m, H-6a), 3.83 (3H, s, OCH_3), 3.73 (3H, s, OCH_3), 3.1–3.2 (2H, m, H-4″) and 1.73 (3H, s, H-8′); ^{13}C-NMR (400 MHz,), δ (ppm): 191.1 (C-12), 168 (C-9), 157.7 (C-7a), 151.1 (C-3), 148.4 (C-4a), 142.9 (C-6′), 142.7 (C-2), 130.1 (C-11), 118.8 (C-11a), 113.9 (C-8), 112.7 (C-7′), 109.4 (C-1a), 108.8 (C-1), 105.3 (C-10), 101.1(C-4), 88 (C-5′), 76.1 (C-6a), 67.6 (C-12a), 63.8 (C-6), 55.9, 56.4 (OCH_3), 31.1(C-4″) and 17.1 (C-8′); MS: m/z (rel int., %): 410 (M^+, 1.5%), 392 (0.3%), 208 (100%), 193 1.1%), 181 (0.6%), 165 (1.0%).

2.4. Minimum inhibitory concentration (MIC)

The MIC values of compounds **1**–**6** were determined using a reported procedure with slight modification (Ieven et al., 1979). Varying concentrations (200, 150, 100, 50, 25 and 12.5 mg/ml) of samples were prepared. 0.1 ml of each sample was added to 9 ml nutrient broth containing 0.1 ml of standardised test organism (bacterial cells). The tubes were incubated at 37° C for 24 h. Positive controls were set up by using DMSO. Ten milligrams per millilitre of the isolated compounds **1**–**6** were assayed as described above. The tube with the least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentration (MIC). Minimum fungicidal concentration (MFC) values were recorded as the concentrations of the lowest clear wells of each extract. The assay was carried out in duplicate.

2.5. Statistical analysis

All data were expressed as the mean ± S.E.M., data were subjected to two-way ANOVA followed by Student's t-test, using Microsoft Excel* and Statistical* computer software packages. Difference in mean was considered significant when $P \leqslant 0.05$.

3. Results and discussion

3.1. Compound *1* (neoraudiol)

Compound 1 was eluted from column with hexane/Et2O (3:1, vol/vol) and recrystallised from hexane–EtOAc (3:1, vol/vol) as light-green powder (65 mg), mp 205–206° C. It gave a positive ferric chloride reaction, indicative of its phenolic nature. Its EIMS spectrum gave a molecular ion peak at m/z 294, corresponding to the molecular formula C17H10O5. Infra red absorption bands at 3781.2, 1665.7, 1593.8 cm−1 indicated the presence of hydroxyl, conjugated carbonyl and arene C=C functional groups, respectively. The UV spectrum of **1** displayed two maximum bands [λ_{max} (EtOH)] at 389.8 and 323 nm, characteristic of α,β-unsaturated ketones (Chun-Ru et al., 2010). The ^{1}H-NMR spectrum of **1** (Table 1) gave resonances of 8 aromatic protons at δ 8.49 [1H, s, H-5], δ 8.25 [1H, s, H-2], δ 7.94 [1H, d, J = 2 Hz H-2″], δ 7.76 [1 H, s, H-8], δ 7.10 [1H, d, J = 8 Hz, H-5′], δ 7.05 [1H, dd, J = 2, 1.6 Hz H-3″], δ 6.41 [1H, dd, J = 8, 2 Hz H-6′] and δ 6.38 [1H, d, J = 2 Hz, H-2′]. The two proton signals at δ 7.05 [1H, dd, J = 2, 1.6 Hz, H-3″] and δ 7.94 [1H, d, J = 2 Hz H-2″] were

Table 1 ^{1}H-NMR spectroscopic data of compound **1**.

Positions	δ_H	HMBC	NOESY
2	8.25 s	C-4, 4a, 6, 8	
5	8.49 s	C-2, 4, 6, 7, 1′	
8	7.76 s	C-2, 2″, 6	H-2′
2′	6.38 d (2)	C-3, 4′, 5′, 6′	H-5′
5′	7.10 d (8)	C-3,1′, 2′, 3′,	H-2′
6′	6.41 dd (8, 2)	C-3, 2′, 3′	H-5′
2″	7.94 d (2)	C-6, 8, 8a	H-3″
3″	7.05 dd (2, 1.6)	C-4, 4a	H-2″
–OH	4.81 s, br		H-5′, 6′

^{1}H-NMR, HMBC and NOESY spectral data were acquired in CD_3OD at 400 MHz. Chemical shift values are shown in the δ scale (ppm), with coupling constants (J, Hz) in parentheses, s = singlet, d = doublet, dd = doublet of doublet.

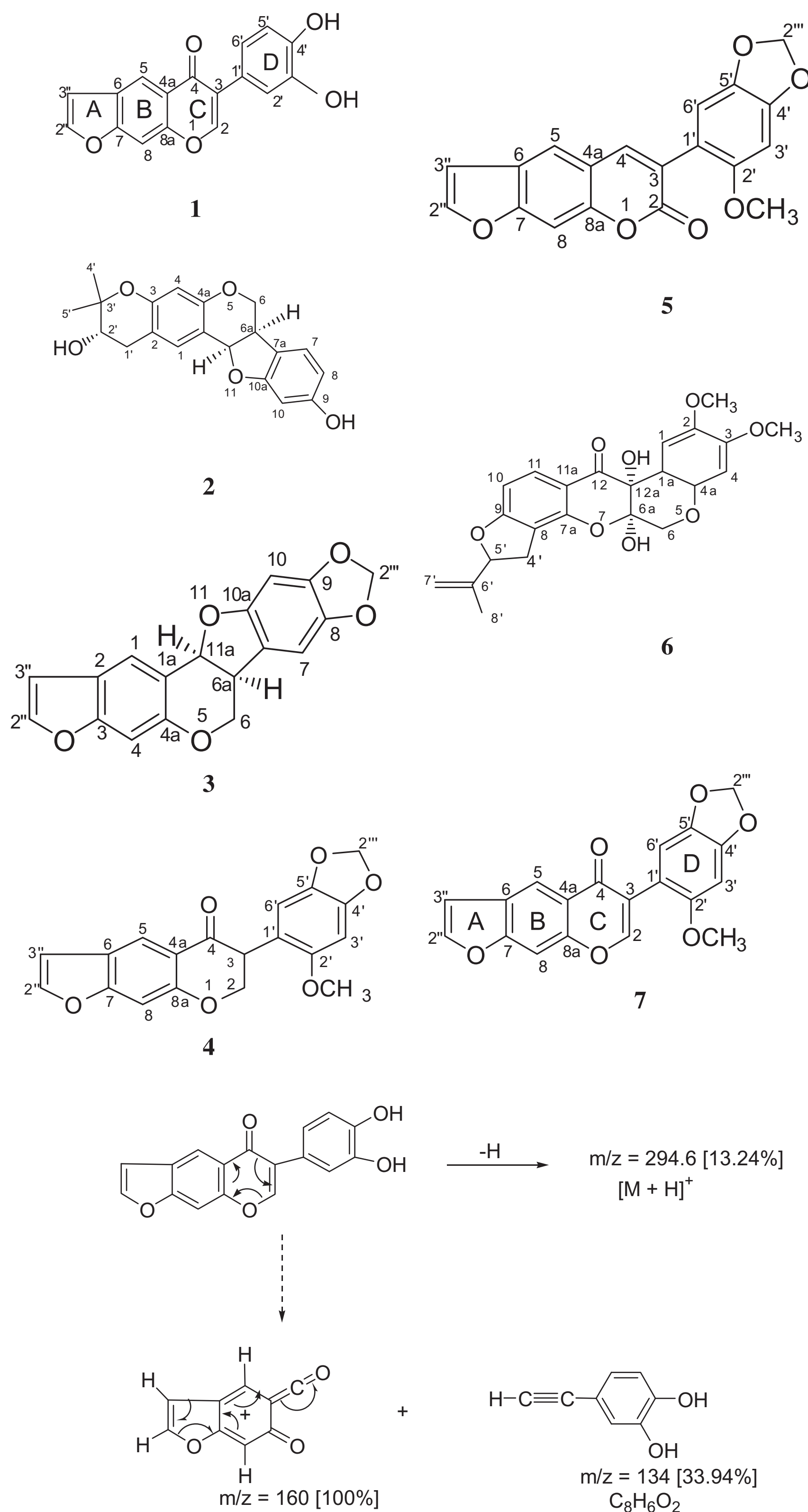

Figure 1 Fragmentation pattern of **1**.

assigned to the olefinic protons of the benzofuran skeleton by an analysis of its NOESY 2D NMR data. The COSY spectrum of **1** indicated two isolated spin system for H-5′/H-6′ and H-2″/H-3″. The ^{13}C-NMR and DEPT spectra of **1** exhibited eight methine and nine quaternary carbons, including a carbonyl carbon at δ 180 ppm [C-4]. The 8 methine carbons resonates at δ 157.4 [C-2], δ 149.7 [C-2″], δ 133.1 [C-2′], δ 120 [C-5], δ 108.3 [C-6′], δ 106.1 [C-3″], δ 104.5 [C-5′], and δ 100.9 [C-8].

Table 2 Minimum inhibitory concentration of compounds isolated from *N. mitis*.

Compounds	Micro-organisms/MIC (mg/ml) values						
	S. A.	B. S.	E. C.	P. A.	S. T.	C. A.	A. N.
Neoduline	NA	NA	75.1 ± 0.2	NA	66.3 ± 1.9	11.5 ± 0.1	11.2 ± 0.4
Neotenone	55.1 ± 0.8	45.2 ± 0.3	NA	NA	NA	11.2 ± 0.2	22.2 ± 0.1
Pachyrrhizine	NA	69.1 ± 0.8	NA	80.2 ± 0.2	NA	NA	NA
12a-hydroxy rotenone	45.7 ± 1.3	60.1 ± 0.9	NA	65.5 ± 0.5	35.1 ± 0.4	15.1 ± 0.1	11.2 ± 0.4
Neoraudiol	35.4 ± 0.6	15.1 ± 0.5	23.4 ± 0.2	17.4 ± 0.6	17.4 ± 0.7	17.5 ± 0.9	17.3 ± 0.1
Rautandiol A	NA	20.2 ± 0.6	NA	55.1 ± 0.8	NA	10.2 ± 0.1	17.2 ± 0.3
Gentamicin[a]	12.1 ± 0.2	16.9 ± 0.3	17.5 ± 0.4	16.1 ± 0.2	14.1 ± 0.4	–	–
Thioconazole[b]	–	–	–	–	–	20.1 ± 0.2	18.1 ± 0.2

Concentration of reference drugs and isolated compounds is 20 mg/ml. NA = not active.
S. A. = *Staphyllococcus aureus*, B. S. = *Bacillus subtilis*, E. C. = *Eschericia coli*, P. A. = *Pseudomonas aueriginosa*, S. T. = *Salmonella typhii*, C. A. = *Candida albicans*, A. N. = *Aspergillus niger*.
[a] Reference antibacterial drug.
[b] Reference antifungal drug.

The nine quaternary carbons signals are at δ 180.0 [C-4], δ 160 [C-8a], δ 159 [C-7], δ 123.5 [C-3], δ 122 [C-1′], δ 119.4 [C-4a], δ 119 [C-6], δ 107.9 [C-4′] and δ 104.1[C-3′]. A complete assignment of the ^{1}H- and ^{13}C-NMR spectra of **1** was based on the HMBC, HMQC, COSY and NOESY experiments. The location of the carbonyl group was confirmed by the analysis of its HMBC spectrum. In the HMBC spectrum, there were correlations between the proton signals at δ 8.25 [1H, s, H-2], δ 8.49 [1H, s, H-5] and the carbon resonance at δ 180 ppm. A comparison of the ^{1}H- NMR signals (δ_H 8.49, 8.25, 7.94, 7.76 and 7.05 ppm) in rings A, B and C with those reported for dehydroneotenone (7) in *N. mitis* are the same (Luc et al., 1987), except the difference in the substitution pattern in the aromatic ring D. The substitution pattern in ring D was further analysed by its ^{1}H-NMR, HMBC and NOESY spectroscopic data. An ortho coupled doublets observed in the ^{1}H-NMR at 7.10 (d, J = 8 Hz), 6.41 (1H, dd, J = 8, 2 Hz,); and a meta coupled doublet at 6.38 (d, J = 2 Hz) were assigned to H-5′, H-6′ and H-2′, respectively, on the basis of NOESY experiment (Table 1). The correlations observed in HMBC spectrum of **1** indicated that the two –OH groups are assigned to C-3′ and C-4′. The dihydroxyl moieties in ring D were further confirmed by the fragment ion in EIMS at *m/z* 134 [(33.94%), $C_8H_6O_2$] (Fig. 1). The carbon signal at 133.1 ppm was assigned to C-2′ based on the correlations from the proton signals located at δ 8.25 [1H, s] to the carbon signals at δ 123.5 [C-3] and δ 107.9 [C-4′], this placed the aromatic proton at C-2′. In addition, the remaining aromatic protons at positions 5′ and 6′ were further supported by HMBC correlation between δ 7.10 and δ 6.41 to δ 123.5 [C-3]. On the basis of the spectral data outlined above, the structure of the compound **1** was deduced as neoraudiol, a new isoflavonoid isolated from *N. mitis*.

3.2. Compound 2 (rautandiol A)

Compound **2** was eluted from column with 1:1 hexane–Et_2O as a grey solid (83 mg, mp 210–211° C, lit mp 210–211° C). The structure of **2** was confirmed by comparison of its 1D and 2D NMR and EIMS spectroscopic data with those found in the literature for a pterocarpan, rautandiol A, previously reported from *N. mitis* (Luc et al., 1987).

3.3. Compound 3 (neoduline)

Compound **3** was eluted with hexane/Et_2O (3:1, vol/vol) to afford a white powdery solid [95 mg, mp 216–217° C, lit mp 217° C]. Its ^{1}H-, ^{13}C-NMR and EIMS spectral data are consistent to that of neoduline, a pterocarpan, previously reported as constituents of *N. mitis* (Luc et al., 1987).

3.4. Compound 4 (neotenone)

Compound **4** was eluted with 9:1 hexane–Et_2O as pure greenish-white solid [100 mg, mp 157–158° C, lit mp 156° C]. ^{1}H- and ^{13}C-NMR spectra of the compound were similar to those reported for neotenone, a flavone previously isolated from *N. mitis* (Luc et al., 1987).

3.5. Compound 5 (pachyrrhizine)

Compound **5** was eluted from column with 7:3 hexane–Et_2O and recrystallised from hexane/EtOAc solvent mixture (7:3, vol/vol) to afford a yellow solid [65 mg, mp 206–207° C, lit mp 204–205° C]. Compound **5** was identified as pachyrrhizine, on the basis of ^{1}H-NMR and GC–MS spectral data, and by comparison with data reported in the literature (Luc et al., 1987).

3.6. Compound 6 (12a-hydroxy rotenone)

Compound **6** was eluted from column with 3:2 hexane–Et_2O as a yellow solid (500 mg, mp 86–87° C; lit mp 87–88° C). The NMR and MS spectral data of **6** established the structure as 12a-hydroxy rotenone, a pterocarpan reported from the tuberous root of *N. mitis* (Luc et al., 1987).

Compounds **1**–**6** were assayed for antimicrobial activity against four bacteria and two fungi by determining the MIC. The results are shown in Table 2. Compounds **1**–**6** displayed varying degree of antimicrobial activities on the pathogenic strains. Among all the six compounds neoraudiol (**1**) displayed highest antimicrobial activity on *B. subtilis* (MIC 15.10 ± 0.5 mg/ml) and *S. typhii* (MIC 17.30 ± 0.7 mg/mL). Compound **1** equally demonstrates stronger antifungal activity against *C. albicans* (MIC 17.45 ± 0.9 mg/ml) and *A. niger* (MIC 17.3 ± 0.1). Rautandiol A (**2**) exhibits moderate antimi-

crobial activity, while other compounds are relatively less active. The observed activity of compound **1** can be traced to the phenolic nature of polyphenols. Previous report had attributed high activity of phenolic compounds to the presence of phenolic (–OH) group Aziz et al., 1998). The hydroxyl group is reactive and forms hydrogen bonds with active sites of enzymes. High activity of phenolic compounds on different pathogenic strains has been reported (Leticia et al., 2008). The intrinctly high antimicrobial activity of compound **1** is consistent with earlier report. The lipophilic nature of compound **1** could be attributed for the high antifungal activity, as it allows it to penetrate more easily through the fungus membrane. The antibacterial and antifungal activities exhibited by the extracts and compounds isolated from *N. mitis* confirmed its use in traditional medicine for the treatment of syphilis and skin infections.

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