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Synthesis of 4',5,7-trihydroxyflavanone and 3',4',5,7-tetrahydroxyflavanone and antioxidant activity

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ABSTRACT

Natural flavonoids, 4',5,7-trihydroxyflavanone and 3',4',5,7-tetrahydroxyflavanone were synthesised *via* their respective chalcone. The initial step was to synthesise derivatives of 2-hydroxyacetophenone and benzaldehyde by protecting the phenolic hydroxyl groups. The chalcone was synthesised by Claisen-Schmidt condensation. Acid hydrolysis and subsequent treatment with sodium acetate of 2'-hydroxy-4,4'-6'-tris(methoxymethoxy)chalcone and 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone, gave 4',5,7-trihydroxyflavanone and 3',4',5,7-tetrahydroxyflavanone, respectively. 3',4',5,7-Tetrahydroxyflavanone was found to be more potent as an antioxidant agent than 4',5,7-trihydroxyflavanone with 83.11% inhibition and SC₅₀ 8.57 µg/mL in the radical scavenging activity by ESR method.

| 4',5,7-Trihydroxyflavanone | 3',4',5,7-Tetrahydroxyflavanone | Chalcones | Antioxidant | ESR |

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1. INTRODUCTION

Flavonoids are a large group of polyphenolic compounds possessing a basic flavan nucleus with two aromatic rings (the A and B rings) interconnected by a three-carbon-atom heterocyclic ring (the C ring). The most widespread flavonoids contain a double bond between C-2 and C-3 and a keto function at C-4 of ring C [1]. Flavonoids represent one of the largest and most diverse classes of plant secondary metabolites which are naturally present in vegetables, fruits, and beverages. They possess a wide variety of biological activities including anti-inflammatory, antioxidant, antimutagenic, anti-HIV, vasodilator, anticancer, and cardiovascular effects [2]. The antioxidant activities of flavonoids have been evaluated against reactive oxygen species like 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical using the UV technique [3]. The application of the Electron Spin Resonance (ESR) technique to test the direct scavenging activities of flavonoids was first published by Goa *et al.* in 1999 [4].

The interest to study the antioxidant activity of the flavonoids has prompted us to synthesise naringenin (1) and eriodictyol (2); two hydroxylated flavanones abundant in grape fruit and lemon, respectively [5,6].

2. EXPERIMENTAL

2.1 Materials, method and instruments

Melting points were recorded on a Leica Galen III Kofler micro melting point apparatus and were uncorrected. Infrared (IR) spectra were recorded on Shimadzu 8000 or Perkin-Elmer series 1600 spectrometers as thin film for liquid samples or KBr pellet for solid samples. Mass spectral data were obtained from Kent Mass Spectrometry Service, UK. ¹H and ¹³C NMR spectra (300 and 75 MHz, respectively) were recorded on a Bruker Avance 300 Spectrometer using CDCl₃ and DMSO as solvent. Reactions were monitored by thin-layer chromatography (tlc) carried out on 0.2 mm Merck pre-coated silica gel plates (60 F₂₅₄).

2.2 4,5,7-trihydroxyflavanone (1)

4-Methoxymethoxybenzaldehyde (5). To a suspended solution of 4-hydroxybenzaldehyde (3) (300 mg, 2.450 mmole) in CH₂Cl₂ (10 mL) at 0°C, *N,N*-diisopropylethylamine (8 mL) was added, this was followed by the addition of DMAP (52.8 mg, 0.435 mmole) and the reaction mixture was stirred for 15 min. MOMCl (216 mg, 2.705 mmole) was then added drop wise at 0°C, and the mixture was stirred for 15 min, after which time the temperature was increased to room temperature with stirring overnight. The reaction mixture was then poured into water

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and extracted with CHCl_3 . The organic layer was washed with water and brine, followed by drying over anhydrous MgSO_4 . The solvent was evaporated under reduced pressure, and the resulting syrup was chromatographed on a silica gel column (PE: EtOAc, 9: 1) to give 4-methoxymethoxybenzaldehyde (5) (377 mg, 93.0%) as a colourless liquid with R_f 0.56 (PE; EtOAc, 1:4); IR ν_{max} (film) cm^{-1} : 1689 (C=O), 1600 and 1507 (aromatic C=C), 1311 (C-O); NMR δ_{H} (CDCl_3) ppm: 3.49 (3H, s, -OCH₃), 5.26 (2H, s, -OCH₂-), 7.16 (2H, d, J = 6.9 Hz, H-3 and H-5), 7.85 (2H, d, J = 6.9 Hz, H-2 and H-6) and 9.91 (1H, s, -CHO).

2-Hydroxy-4,6-bis(methoxymethoxy)acetophenone (8). To a suspended solution of 2,4,6-trihydroxyacetophenone (7) (0.5 g, 2.976 mmole) in CH_2Cl_2 (10 mL) at 0°C , *N,N*-diisopropylethylamine (6 mL) was added, this was followed by the addition of DMAP (35.2 mg, 0.290 mmole) and the reaction mixture was stirred for 15 mins. MOMCl (175 mg, 2.170 mmole) was then added drop wise at 0°C , and the mixture was stirred for 15 min, after which time the temperature was increased to room temperature with stirring overnight. The reaction mixture was then poured into water and extracted with CHCl_3 (2 x 20 mL). The organic layer was washed with water and brine, followed by drying over anhydrous MgSO_4 . The solvent was evaporated under reduced pressure, and the resulting syrup was chromatographed on a silica gel column (PE : EtOAc, 9:1) to give 2-hydroxy-4,6-bis(methoxymethoxy)acetophenone (8) (0.41 g, 53.8%) as a colourless oil with R_f 0.69 (PE; EtOAc, 3: 2); IR ν_{max} (film) cm^{-1} : 3443 (O-H), 1623 (C=O), 1599 and 1434 (C=C aromatic); NMR δ_{H} (CDCl_3) ppm: 2.67 (3H, s, -COCH₃), 3.47 (3H, s, -OCH₃), 3.53 (3H, s, -OCH₃), 5.19 (2H, s, -OCH₂), 5.27 (2H, s, -OCH₂-), 6.26 (1H, d, J = 2.2 Hz, H-5), 6.28 (1H, d, J = 2.2 Hz, H-3), 13.71 (1H, s, -OH).

2'-Hydroxy-4,4',6'-tris(methoxymethoxy)chalcone (9). To a solution of 2-hydroxy-4,6-bis(methoxymethoxy)acetophenone (8) (141 mg, 0.553 mmole) in EtOH (5 mL) was added 4-methoxymethoxybenzaldehyde (5) (101 mg, 0.6084 mmol) followed by addition of NaOH (30%) (0.9 mL). The reaction mixture was left at room temperature for overnight. After that, the reaction mixture was poured into iced water and acidified with HCl (10%). The reaction mixture was then poured into water and extracted with EtOAc. The organic layer was washed with water and brine, followed by drying over anhydrous MgSO_4 and the solvent was evaporated under reduced pressure. The resulting syrup was chromatographed on a silica gel column (PE: EtOAc, 9: 1) to give 2'-hydroxy-4,4',6'-tris(methoxymethoxy)chalcone (9) (106 mg, 45.0%) as yellow crystals, m.p. $46-48^\circ\text{C}$ and R_f 0.25 (PE: EtOAc, 3: 2); IR ν_{max} (KBr) cm^{-1} : 1600 and 1462 (C=C aromatic), 1622 (C=O), 1155.0 (C-O); NMR δ_{H} (CDCl_3) ppm: 3.45-3.55 (9H, 3x -OCH₃), 5.17- 5.27 (6H, 3x -OCH₂), 6.26 (1H, d, J = 2.1 Hz, H-3'), 6.33 (1H, d, J = 2.1 Hz, H-5'), 7.08 (2H, d, J = 9.0 Hz, H-3 and H-5), 7.57 (2H, d, J = 9.0 Hz, H-2 and H-6), 7.82 (1H, d, J = 10.2 Hz,

H- α), 7.87 (1H, d, J = 10.2 Hz, H- β), 13.9 (1H, s, -OH); EIMS: m/z 404 [M^+ , $\text{C}_{21}\text{H}_{24}\text{O}_8$, (10)].

4',5,7-Trihydroxyflavanone (1). To 2'-hydroxy-4,4',6'-tris(methoxymethoxy)chalcone (9) (113 mg, 0.279 mmol) in MeOH (6 mL) was added HCl (10%) (1 mL) and the mixture was refluxed for 1.5 h. Then NaOAc (406 mg, 4.850 mmole) was added and the resulting mixture was refluxed for 3 h. The mixture was cooled and H_2O (25 mL) was added and extracted with EtOAc (30 mL x 2), dried over anhydrous MgSO_4 , filtered, and evaporated to dryness to yield the targeted compound, 4',5,7-trihydroxyflavanone (1) (64.5 mg, 85.1%) as a brown solid with m.p. $250-252^\circ\text{C}$ (lit. [7] $248-251^\circ\text{C}$) and R_f 0.33 (PE: EtOAc, 1: 4); IR ν_{max} (KBr) cm^{-1} : 3300-3200 (-OH), 1636 (C=O), 1600.0 and 1460 (C=C aromatic) and 1158 (C-O); UV λ_{max} (MeOH) nm: 330 (shoulder), 289; NaOH; 324, 245; AlCl_3 ; 379, 310; AlCl_3/HCl ; 379, 310; NaOAc; 324; NaOAc/ H_3BO_3 ; 331 (sh), 289; NMR δ_{H} (DMSO) ppm: 2.67 (1H, dd, J = 17.1 and 3.0 Hz, H-3), 3.26 (1H, dd, J = 17.1 and 12.6 Hz, H-3), 5.43 (1H, dd, J =12.6 and 3.0 Hz, H-2), 5.84 (2H, s, H-6 and H-8), 6.69 (2H, d, J = 6.3 Hz, H-2' and H-6'), 7.30 (2H, d, J = 6.3 Hz, H-3' and H-5'), 9.60 (1H, s, -OH), 10.81 (1H, s, -OH), 12.13 (1H, s, -OH); CIMS: m/z 272 [M^+ , $\text{C}_{15}\text{H}_{12}\text{O}_5$, (100)].

2.3 3',4',5,7-tetrahydroxyflavanone (2)

3,4-Bis(methoxymethoxy)benzaldehyde (6). To a suspended solution of 3,4-dihydroxybenzaldehyde (4) (100 mg, 0.724 mmole) in CH_2Cl_2 (10 mL) at 0°C , *N,N*-diisopropylethylamine (3 mL) was added, this was followed by addition of DMAP (17.6 mg, 0.145 mmole) and the reaction mixture was stirred for 15 mins. MOMCl (175 mg, 2.170 mmole) was then added drop wise at 0°C , and the mixture was stirred for 15 min, after which time the temperature was increased to room temperature with stirring overnight. The reaction mixture was then poured into water and extracted with CHCl_3 (2 x 20 mL). The organic layer was washed with water and brine, followed by drying over anhydrous MgSO_4 . The solvent was evaporated under reduced pressure, and the resulting syrup was chromatographed on a silica gel column (PE : EtOAc, 9: 1) to give 3,4-bis(methoxymethoxy)benzaldehyde (6) (139 mg, 75.1%) as a brown liquid with R_f 0.44 (PE: EtOAc, 4:1); IR ν_{max} (film) cm^{-1} : 1690 (C=O), 1595 and 1444 (C=C), 1262 (C-O); NMR δ_{H} (CDCl_3) ppm: 3.53 (6H, s, -OCH₃), 5.31 (2H, s, -OCH₂), 5.34 (2H, s, -OCH₂-), 7.29 (1H, d, J = 8.2 Hz, H-5), 7.52 (1H, dd, J = 1.8 and 8.2 Hz, H-6), 7.69 (1H, d, J = 1.8 Hz, H-2), 9.87 (1H, s, -CHO).

2'-Hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone (10). To a solution of 2-hydroxy-4,6-bis(methoxymethoxy)acetophenone (8) (111 mg, 43.440 mmole) in EtOH (5 mL) was added 3,4-bis(methoxymethoxy)benzaldehyde (6) (108 mg, 47.790 mmole) followed by addition of KOH (40%) (0.9 mL). The reaction mixture was left at room temperature for overnight. After that, the reaction mixture was poured into ice water and acidified with HCl (10%). The reaction mixture was

then poured into water and extracted with CH_2Cl_2 (3 x 20 mL). The organic layer was washed with water and brine, followed by drying over anhydrous MgSO_4 and the solvent was evaporated under reduced pressure to produce syrup. The resulting syrup was chromatographed on a silica gel column (PE: EtOAc, 9: 1) to give 2'-hydroxy-3',4',4,6-tetrakis(methoxymethoxy)chalcone (10) (104 mg, 51.6%) as yellow crystals, m.p. 88-90°C (lit [8] m.p. 91-92 °C) and R_f 0.25 (PE: EtOAc, 3: 2); IR ν_{max} (KBr) cm^{-1} : 1625 (C=O), 1581 and 1353 (C=C olefinic), 1161 (C-O); NMR δ_{H} (CDCl_3) ppm: 3.55 (12H, m, -OCH₃), 5.25 (8H, m, -OCH₂), 6.29 (1H, d, J = 2.2 Hz, H-3'), 6.33 (1H, d, J = 2.2 Hz, H-5'), 7.19 (1H, d, J = 8.4 Hz, H-6), 7.23 (1H, d, J = 8.4 Hz, H-5), 7.53 (d, J = 1.5 Hz, H-2), 7.56 (1H, d, J = 15.6 Hz, H- α), 7.88 (1H, d, J = 15.6 Hz, H- β), 13.9 (1H, s, -OH); MS: m/z 464 [M^+ , $\text{C}_{23}\text{H}_{28}\text{O}_{10}$, (10)].

3',4',5,7-Tetrahydroxyflavanone (2). To 2'-hydroxy-3',4',4,6-tetrakis(methoxymethoxy)chalcone (10) (0.05 g, 0.108 mmole) in MeOH (6 mL) was added HCl (1 mL) and the mixture was refluxed for 1.5 h. Then NaOAc (0.18 g, 2.150 mmole) was added and then the resulting mixture was refluxed for 3 h. The mixture was cooled and H_2O (25 mL) was added and extracted with EtOAc (30 mL x 2), dried over anhydrous MgSO_4 , filtered, and evaporated to dryness to afford the desired product, 3',4',5,7-tetrahydroxyflavanone (2) in 74.8% yield as brown solid with m.p 194-196°C (lit. [9] 196-197°C) and R_f 0.27 (PE: EtOAc, 1: 4); IR ν_{max} (KBr) cm^{-1} : 3411 (OH), 1655 (C=O), 1608 (aromatic C=C) and 1264 (C-O); UV λ_{max} (MeOH) nm: 331 (shoulder), 288; NaOH; 322, 249; AlCl_3 ; 367 (sh), 309; AlCl_3/HCl ; 369 (sh), 309; NaOAc; 325, 288 (sh); NaOAc/ H_3BO_3 ; 323 (sh), 288; NMR δ_{H} (DMSO) ppm: 2.67 (1H, dd, J = 17.1 and 3.0 Hz, H-3a), 3.19 (1H, dd, J = 17.1 and 12.4 Hz, H-3b), 5.37 (1H, dd, J = 12.4 and 3.0 Hz, H-2), 5.87 (2H, s, H-6 and H-8), 6.73 (2H, s, H-5' and H-6'), 6.87 (1H, s, H-2'), 9.04 (1H, s, -OH), 9.09 (1H, s, -OH), 10.80 (1H, s, -OH), 12.13 (1H, s, -OH); MS: m/z 287 [$\text{M}-1$, $\text{C}_{15}\text{H}_{12}\text{O}_6$, (7)].

2.4 Antioxidant screening (free radical scavenging activity)

DPPH radical scavenging using ESR (Electron Spin Resonance) was carried out according to the method described by Ohtani *et al.* [10] with some minor modifications. The ethanolic solution of the test sample 200 μL (1mg/mL) was added to 200 μL of DPPH (0.25 mM) in ethanol solution. After shaking vigorously for 10 sec, the solution was transferred to a flat cell. The ESR spectra were recorded after 40 sec. The condition of ESR spectrometer were set at room temperature, power 1mW, magnetic field $336.000 \pm 5\text{mT}$, field modulation width 0.5 mT, sweep time 30 sec and time constant 0.03 sec. The scavenging effect of DPPH was calculated by the following formula:

$$\text{Percent Scavenging (\%)} = \frac{\text{PH DPPH} - \text{PH Sample}}{\text{PH DPPH}} \times 100$$

PH= Peak height of the third and the fifth line signals of DPPH radical.

The SC_{50} value was determined as the concentration of each sample required to give 50% of scavenging of DPPH. All test and analyses were run in triplicates.

3. RESULTS & DISCUSSION

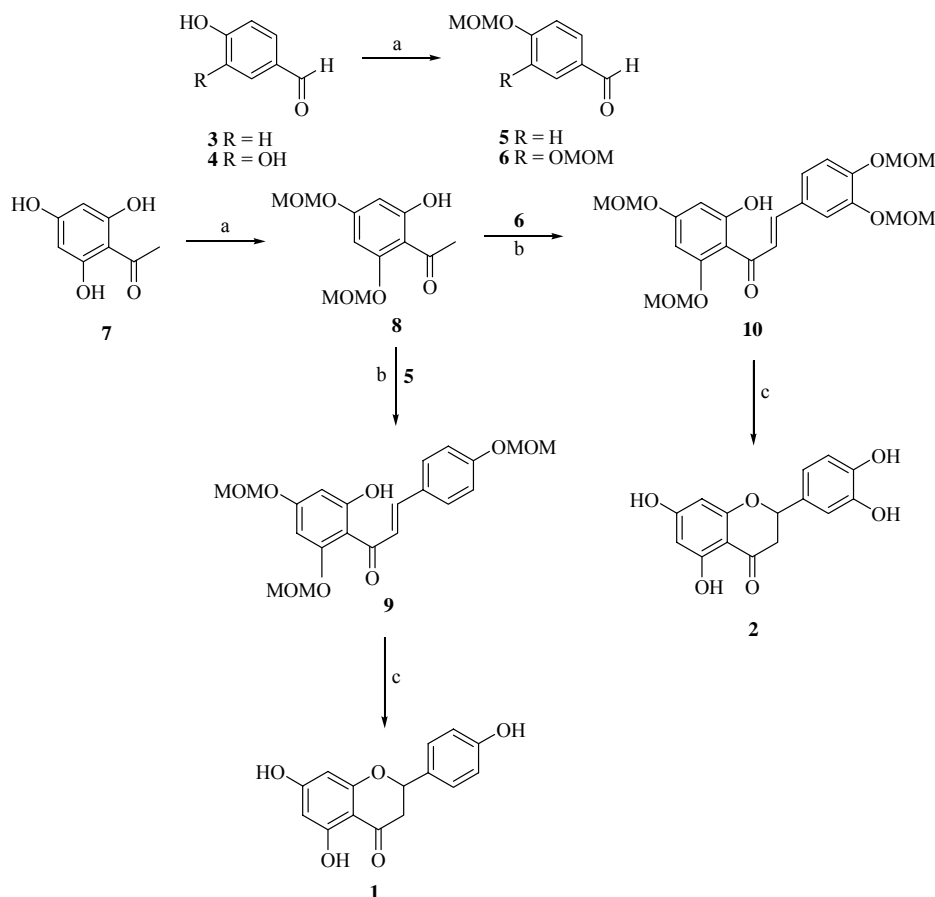
3.1 Synthesis 4',5,7-trihydroxyflavanone (1)

Scheme 1.0 illustrates the detailed synthetic route to (1) and (2). For both routes, the hydroxyl groups of benzaldehydes (3 and 4) were protected by converting to the MOM derivatives, (5) and (6), respectively. 4',5,7-Trihydroxyflavanone (1) or naringenin is the main flavonoid compound found in grapefruit and has the potential to act as an antitumorogenic [11] and anti-inflammatory agents [5]. The synthesis of (1) was initiated by the preparation of protected benzaldehyde (5). 4-Methoxymethoxybenzaldehyde (5) was isolated in excellent yield, 93.0% as a colourless liquid and has an IR spectrum with absorption bands at 1689 cm^{-1} (C=O) and 1600 and 1507 cm^{-1} (C=C aromatic). The presence of -MOM protecting group was confirmed by the protons resonances at δ 3.49 (3H, s, -OCH₃), 5.26 (2H, s, -OCH₂-) in the ^1H NMR spectrum. 2,4,6-Trihydroxyacetophenone (7) was converted to its methoxymethylether derivative. A reaction of (7) with methoxymethylchloride (MOMCl) in the presence of dimethylaminopyridine (DMAP) and *N,N*-ethyl-diisopropylamine in dry CH_2Cl_2 afforded (8) as colourless oil in 53.8% yield. The Claisen-Schmidt condensation of (5) and (8) under basic condensation afforded 2'-hydroxy-4,4',6'-tris(methoxymethoxy)chalcone (9). The EIMS of (9) gave a molecular ion peak at m/z 404 corresponding to $\text{C}_{21}\text{H}_{24}\text{O}_8$. The absence of a hydroxyl absorption band was noticed in the IR spectrum, but its presence was later deduced from a broad band phenolic proton signal at δ 13.9 in the ^1H NMR spectrum. A set of mutually coupled protons at δ 7.82 (1H, d, J = 10.2 Hz, H- α), 7.87 (1H, d, J = 10.2 Hz, H- β) confirmed the formation of chalcone (9).

Deprotection of the MOM group of chalcone (9) was achieved by using HCl (10%) in MeOH and cyclisation was carried out in NaOAc/MeOH to furnish (1) as brown solids in 85.1% yield. The IR spectrum displayed a broad absorption band at $3300\text{-}3200\text{ cm}^{-1}$ attributable to the hydroxyl group. The UV spectrum showed characteristics absorption for a flavanone at 330 (band I) and 289 (band II) nm. A bathochromic shift of 35 nm was noted for band II after an addition of NaOH and NaOAc shift reagents, indicating a 5,7-OH flavanone [12]. The CIMS spectrum showed a molecular ion peak at m/z 272 which was in accordance with the molecular formula $\text{C}_{15}\text{H}_{12}\text{O}_5$. The ^1H NMR spectrum displayed typical ABX splitting pattern for a flavanone at δ 2.67 (1H, dd, J = 17.1 and 3.0 Hz, H-3a), 3.26 (1H, dd, J = 17.1 and 12.6 Hz, H-3b) and 5.43 (1H, dd, J = 12.6 and 2.7 Hz, H-2). The phenolic protons were observed at δ 12.13 (1H, H-5), 10.81 (1H, H-7) and 9.60

(1H, H-4'). The IR spectrum displayed stretching bands for -OH at 3443 cm⁻¹ and a carbonyl at 1623 cm⁻¹. The ¹H NMR spectrum of (8) showed the presence of signals due to methylene protons at δ 3.47 (3H) and 3.53 (3H), which

proved that (7) has been protected. The phenolic proton resonated as a singlet at δ 13.71.



Scheme 1.0: MOMCl, DMAP, Net(*i*-Pr)₂, dry CH₂Cl₂, 24 h, 0°C, rt; (b) KOH 40%, EtOH, rt, 72 h; (c) i. HCl 10%, MeOH; ii. NaOAc, reflux 3 h.

3.2 Synthesis 3',4',5,7-tetrahydroxyflavanone (2)

Protection of (4) with MOMCl yielded 3,4-bis(methoxymethoxy)benzaldehyde (6) in 75.1% yield as a brown liquid. The IR spectrum showed bands for carbonyl (1690 cm⁻¹), aromatic rings (1595 and 1444 cm⁻¹) and C-O (1262 cm⁻¹). The presence of the protecting groups was confirmed by the signals resonated at δ 3.53 (6H, s, -OCH₃), 5.34 (2H, s, -OCH₂) and 5.31 (2H, s, -OCH₂-) in the ¹H NMR spectrum. The signal for the aldehyde proton appeared at δ 9.87.

Condensation of (6) with (8) afforded 2'-hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone (10). The ¹H

NMR spectrum confirmed the formation of the hydroxychalcone moiety with a downfield signal at δ 13.9 for phenolic O-H and a set of *trans*-olefinic protons at δ 7.56 (d, *J* = 15.6 Hz) and 7.88 (d, *J* = 15.6 Hz) for H-α and H-β, respectively. The MS spectrum of (10) displayed a molecular ion peak at *m/z* 464 which was in agreement with a molecular formula C₂₃H₂₈O₁₀.

Acid hydrolysis of chalcone (10) followed by treatment with excessive NaOAc afforded 3',4',5,7-tetrahydroxyflavanone (2) in a high yield (74.8%). The UV spectrum of (2) displayed maximum absorption at 331 (shoulder) and 288 nm indicated the presence of a flavanone skeleton. Addition of NaOH and NaOAc shift reagents

resulted in 34 and 36 nm bathochromic shift of band II, which is typical of 5,7-dihydroxyflavanone [12]. Compound (2) has the characteristic signals for a flavanone at δ 2.67 (1H, dd, $J = 17.1$ and 3.0 Hz, H-3a), 3.19 (1H, dd, $J = 17.1$ and 12.4 Hz, H-3b) and 5.37 (1H, dd, $J = 12.4$ and 3.0 Hz, H-2) in the ^1H NMR spectrum. The carbonyl group at C-4 forms hydrogen bonding with C-5 OH group, as evidenced by a singlet which appeared at δ 12.13. The signals for the remaining phenolic protons were seen at δ 9.04 (1H, H-3'), 9.09 (1H, H-4') and 10.80 (1H, H-7). The aromatic protons of the A-ring were observed overlapping as a singlet at δ 5.87 (2H, s, H-6 and H-8). The three aromatic protons of the B-ring appeared at δ 6.87 (1H, s, H-2') and 6.73 (2H, s, H-5' and H-6'). The MS spectrum showed a molecular ion peak (M-1) at m/z 287 which was in agreement with the molecular formula $\text{C}_{15}\text{H}_{12}\text{O}_6$. The spectroscopic data of (2) were in good agreement with the data of the same compound published by Selenski [13]. Thus the synthesis of 3',4',5,7-tetrahydroxyflavanone or eriodictyol (2) was completed in 15.8% overall yield.

3.3 Electron Spin Resonance (ESR) spectrometry method

In this study, the ESR spectrometry assay has been used to measure the free radical scavenging activity of the antioxidant against DPPH radical. Vitamin C was used as a reference antioxidant and screened for comparison purposes with the synthesised flavanones. Positive DPPH test suggests that the tested compounds are free radical scavengers.

An ESR signal is directly proportional to the number of radicals present. The 0.25 mM DPPH radicals give a typical ESR spectrum as shown in Figure 1.0. The peaks height will be reduced when an antioxidant was added to the ethanolic DPPH solution. The radical scavenging activity of flavanones was expressed by means of SC_{50} which represent the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%.

Table 1.0 : The SC_{50} of the synthesised flavonoids by ESR spectrometry method

Sample	SC_{50} ($\mu\text{g}/\text{mL}$) (mean \pm SD)	Percent inhibition at 15.63 $\mu\text{g}/\text{mL}$ (mean \pm SD)
(1)	77.34 \pm 0.3	31.85 \pm 0.2
(2)	8.57 \pm 0.8	83.11 \pm 2.4
Vitamin C	10.91 \pm 0.4	80.19 \pm 1.9

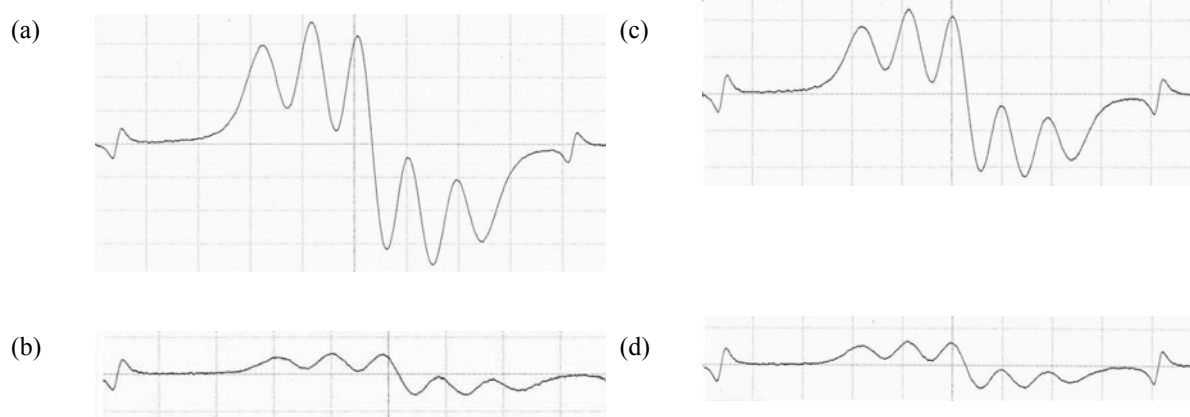


Figure 1.0: ESR spectra of scavenging effects of (b) 3',4',5,7-tetrahydroxyflavanone (2) (percent inhibition: 83.11%), (c) 4',5,7-trihydroxyflavanone (1) (percent inhibition: 31.85%), (d) vitamin C (percent inhibition: 80.19%) against (a) DPPH (0.25 mM) free radical

Table 1.0 shows the activity of the tested compounds. The radical scavenging activity of the active compounds was found to be concentration-dependent manner. The data reveal that 3',4',5,7-tetrahydroxyflavanone (2) possess the strongest activity as free radical scavenger compared with

positive control, vitamin C and flavanone (1), which can be seen from the SC_{50} value of this compound; 8.57 $\mu\text{g}/\text{mL}$ and 83.11% inhibition at concentration 15.63 $\mu\text{g}/\text{mL}$. Figure 1.0 shows the intensities of the DPPH signal at concentration of 15.63 $\mu\text{g}/\text{mL}$ for the flavanoids and vitamin C.

Flavonoids with free hydroxyl groups are known to scavenge free radicals by hydrogen donation. From the results obtained above, the order of effectiveness in scavenging DPPH radicals, also a measure of their antioxidative potentials, is as follows:

3',4',5,7-tetrahydroxyflavanone (**2**) > vitamin C > 4',5,7-trihydroxyflavanone (**1**)

An increase in the number of hydroxyl groups generally enhances the antioxidant activity of the flavonoids [14]. 3',4',5,7-Tetrahydroxyflavanone (**2**) which possess 3',4'-dihydroxyl group in B-ring showed higher antioxidant activity than 4',5,7-trihydroxyflavanone (**1**). The B-ring which has the hydroxyl configuration is the most important determinant of scavenging free radical. It was found that flavonoids with the presence of catechol group in ring B was essential for high antioxidant activity. 4',5,7-Trihydroxyflavanone (**1**) which lacked the *ortho*-dihydroxylation in the B ring reduced the radical scavenging activity.

4. CONCLUSION

4',5,7-Trihydroxyflavanone (**1**) and 3',4',5,7-tetrahydroxyflavanone (**2**) were successfully prepared from their respective chalcones, 2'-hydroxy-4,4',6'-tris(methoxymethoxy)chalcone (**9**) and 2'-hydroxy-3,4,4',6'-tetrakis-(methoxymethoxy)chalcone (**10**), in excellent yields. 3',4',5,7-Tetrahydroxyflavanone (**2**) exhibited high antioxidant activity than the standard, vitamin C with SC₅₀ values of 8.57 µg/mL compared to 4',5,7-trihydroxyflavanone (**1**) with SC₅₀ 77. µg/mL in the free radical scavenging activity against DPPH measured by ESR technique.

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REFERENCES

- [1] T.A Geisman, Organic Chemistry of Secondary Plant Metabolites, Freeman, Cooper and Company, California, 1969, 183-230.
- [2] A. Mohamed Adib, F. Ahmad, and M. S. Idris, J. Chem. Sci., 120 (2008), 1-5.
- [3] F. Neesa, Z. Ismail, N. Mohamed, and M. R. H. Haris, Food Chem., 88 (2004), 243-252.
- [4] Z. Gao, K. Huang, X. Yang, and H. Xu, Biochim. Biophys. Acta., 1472 (1999), 643-650.
- [5] J. A. Manthey, K. Grohman, and N. Guthrie, Curr. Med. Chem., 8 (2001), 135-153.
- [6] K. Minato, Y. Miyake, S. Fukumoto, K. Yamamoto, Y. Kato, Y. Shimomura, and T. Osawa, Life Science, 72 (2003), 1609-1616.
- [7] R. Jain, S. Nagpal, J. Shweta, and S. C. Jain, J. Medicinal and Aromatic Plant Species, 26 (2004), 48-50.
- [8] M. Hu, K. Kransz, J. Chen, G. X. Jun, J. Li, and H. Gelboin, Drug Metabolism and Disposition, 31 (2003), 924-931.
- [9] Y. Xia, Z. Y. Yang, P. Xia, K. F. Bastow, Y. Nakanishi, and K. H. Lee, Bioorg. Med. Chem. Lett, 10 (2000), 699-701.
- [10] Ohtani, N. Gotoh, J. Tanaka, T. Higa, M. A. Gyamfi, and Y. Aniya, J. Nat. Prod, 63 (2000), 676-679.
- [11] S. M. Kuo, Cancer Lett, 110 (1996), 41-48.
- [12] K. R. Markham, Techniques of Flavonoids Identification, Academic Press, London, 1982,
- [13] C. Selenski, and T. R. R. Pettus, Tetrahedron, 62 (2006), 5298-5307.
- [14] G. Cao, E. Sofic, and R. L. Prior, Free Radic, Biol. Med, 22 (1997), 749-760.