VOTE 75148

SYNTHESIS AND CHARACTERIZATION SOME FLAVONOIDS DERIVATIVES

FAREDIAH AHMAD

MUHAMMAD SUM HJ. IDRIS

ADIANA MOHAMED ADIB

**RESEARCH VOTE
75148**

**DEPARTMENT OF CHEMISTRY
FACULTY OF SCIENCE
UNIVERSITI TEKNOLOGI MALAYSIA**

2006

ACKNOWLEDGEMENTS

ABSTRACT

The flavonoids represent an important group of pigment that occurs in the plant kingdom. The flavones, one of the flavonoids, possess interesting biological actions. The antioxidant activity of flavones is reported to be associated with those bearing hydroxyl functions. In the present study, several steps of reaction have been carried out to synthesize the derivatives of luteolin, which are polyhydroxyl flavones. The first step of the reaction was the methylation of 2,4,6-trihydroxyacetophenone with methyl iodide to afford 2-hydroxy-4,6-dimethoxyacetophenone. On aldol condensation of 2-hydroxy-4,6-dimethoxyacetophenone with 3,4-dimethoxybenzaldehyde yielded 3,4,4',6'-tetramethoxychalcone. This was followed by the oxidative cyclisation of chalcone with SeO_2 to give 3',4',5,7-tetramethoxyflavone. The protection of hydroxyl functions of 2,4,6-trihydroxyacetophenone and 3,4-dihydroxybenzaldehyde with methoxy methyl chloride to form 2-hydroxy-4,6-bis(methoxymethyloxy)-acetophenone and 3,4-bis (methoxymethyloxy)-benzaldehyde was carried out. Both compounds were reacted via the aldol condensation to form 3,4,4',6'-tetrakis(methoxymethyloxy)-chalcone. Treatment of 3,4,4',6'-tetrakis(methoxymethyloxy)-chalcone with excessive sodium acetate afforded us 3',4',5,7-tetrahydroxyflavanone or eriodictyol (**39**) as a single product in high yield.The free radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical by synthesized flavonoids using the electron spin resonance (ESR) technique and UV spectrophotometry method showed that 3',4',5,7-tetrahydroxyflavanone is a strong antioxidant. Compounds were characterized by spectroscopic techniques, i.e. infrared (IR), ultraviolet (UV) spectrophotometry, mass spectrometry (MS) and nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR).

ABSTRAK

Flavonoid mewakili kumpulan pigmen yang penting bagi tumbuhan. Flavon yang merupakan salah satu kelas sebatian flavonoid mempunyai pelbagai aktiviti biologi yang menarik. Aktiviti antioksida bagi flavon dilaporkan mempunyai hubungkait dengan kumpulan hidroksil pada sebatian berkenaan. Dalam kajian ini, beberapa langkah tindak balas dijalankan bagi mensintesis terbitan luteolin iaitu flavon polihidroksil. Langkah pertama tindak balas adalah pemetilan 2,4,6-trihidroksiasetofenon dengan metil iodida kepada 2-hidroksi-4,6-dimetoksiasetofenon. Kondensasi Aldol ke atas 2-hidroksi-4,6-dimetoksiasetofenon dengan 3,4-dimetoksibenzaldehid memberikan 3,4,4',6'-tetrametoksikalkon. Ini diikuti dengan pensiklikan oksidatif menggunakan Se$_2$O bagi menghasilkan 3',4'.5.7-tetrametoksiflavon. Kumpulan hidroksil 2,4,6-trihidroksiasetofenon dan 3,4-dihidroksibenzaldehid dengan metoksi metil klorida menghasilkan 2-hidroksi-4,6-bis(metoksimetiloksi)-asetofenon dan 3,4-bis(mtoksimetiloksi)-benzaldehid turut dijalankan. Tindak balas kondensasi Aldol ke atas kedua-dua sebatian ini memberikan 3,4,4',6'-tetrakis(metoksimetiloksi)-kalkon dengan sodium asetat berlebihan menghasilkan 3',4',5,7-tetrahidroksiflavanon atau eriodiktiol (**39**) dengan peratus hasil yang tinggi. Aktiviti pengaut radikal bebas bagi radikal 2,2-difenil-1-pikrilhidrazil (DPPH) oleh flavonoid sintetik menggunakan teknik resonans spin elektron (ESR) dan UL menunjukkan 3',4',5,7-tetrahidroksiflavanon sebagai agen antioksida yang kuat. Semua sebatian dicirikan menggunakan teknik spektroskopi inframerah (IM), ultralembayung (UL), spektrometri jisim (SJ) dan resonans magnet nucleus (RMN ^{1}H dan ^{13}C).

TABLE OF CONTENTS

SYNTHESIS AND CHARACTERIZATION SOME FLAVONOIDS DERIVATIVES

FAREDIAH AHMAD

MUHAMMAD SUM HJ. IDRIS

RESEARCH MANAGEMENT CENTRE
UNIVERSITI TEKNOLOGI MALAYSIA

SYNTHESIS AND CHARACTERIZATION SOME FLAVONOIDS DERIVATIVES

FAREDIAH AHMAD

MUHAMMAD SUM HJ. IDRIS

ADIANA MOHAMED ADIB

DEPARTMENT OF CHEMISTRY
FACULTY OF SCIENCE
**UNIVERSITI TEKNOLOGI MALAYSIA**

**2006**

CHAPTER 1

INTRODUCTION

1.0   Introduction

Flavonoids are a group of common natural occurring polyphenolic compounds that are widely found in the plant kingdom. They occur naturally as plant pigments in a broad range of fruits and vegetables as well as beverages such as tea, red wine, coffee and beer [1]. Many of the known 4000 different flavonoids to date are part of our regular diet [2].

Flavonoids are C_{15} compounds composed of two aromatic rings linked through three carbon bridge with a carbonyl function located at one end of the bridge. They can be subdivided into several classes of flavonoids and flavonoids related compounds such as dihydrochalcone (1), chalcone (2), flavanones (4), isoflavones (5) and aurones (6) [3]. Structure (3), which forms the skeleton of a large class of flavonoids is known as flavone.
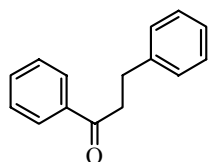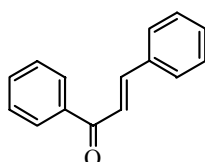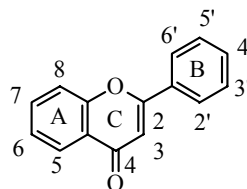
(1)   **(2)**   **(3)**
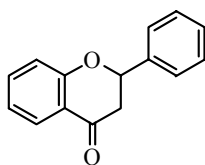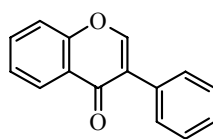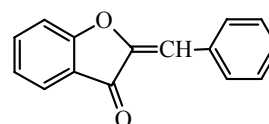
(4)   **(5)**   **(6)**

Flavonoids have gained recent interest because of their broad biological and pharmacological activities. Flavonoids have been reported to exert multiple biological effects including antimicrobial [4], cytotoxicity [5], anti-inflammatory [6] as well as anti-tumor activities [7]. The work of Hertog and co-workers showed the inverse correlation between flavonoids intake and coronary heart disease mortality [8]. Flavonoids have attracted the interest of researchers because they showed promise of being powerful antioxidants which can protect the human body from free radicals [9-11]. Many flavonoids such as quercetin (**7**), luteolin (**8**) and cathechins (**9**), are better antioxidants than the nutrients antioxidants such as vitamin C, vitamin E and β-carotene [12]. The function of an antioxidant is to intercept and react with free radicals at a rate faster than

the substrate. Since free radicals are able to attack at a variety of target including lipids, fats and proteins, it is believed that they may damage organisms, leading to disease, poisoning and including aging [13].

(7)　　　　　　　　　　　　(8)

(9)
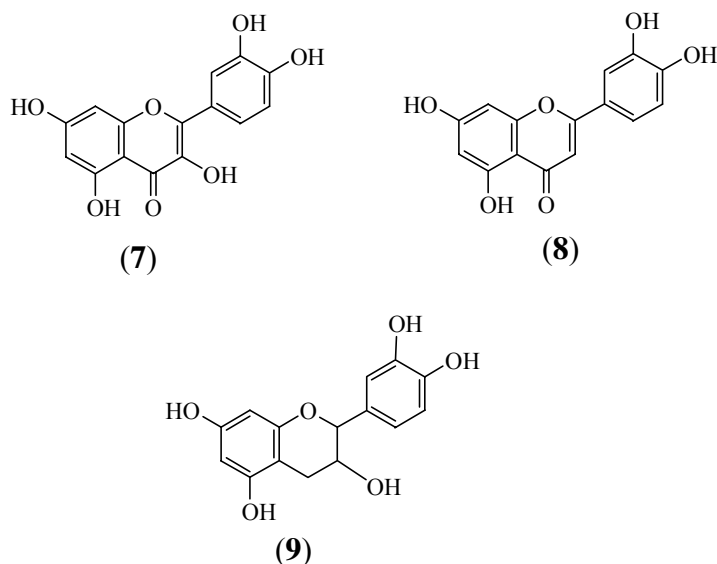
1.1　Synthesis of flavonoids

The interest in the biological properties of flavones has resulted intense synthetic efforts towards the synthesis of various flavones. There are a number of methods reported for the synthesis of flavones.

1.1.1　The Von-Konstanecki Method

This is a general method for synthesizing flavones which involves a reaction of *o*-methoxybenzoate (**10**) and acetophenone in the presence of sodium to form (**11**) as shown in **Scheme 1.1**. The reaction occurs is the Claisen condensation. This is followed by treatment of (11) with an acid to form compound (**12**) followed by elimination of water in order to form the flavone (3) [14].

(10)　　　　　　(11)　　　　　　　　(12)

$-H_2O$

(3)
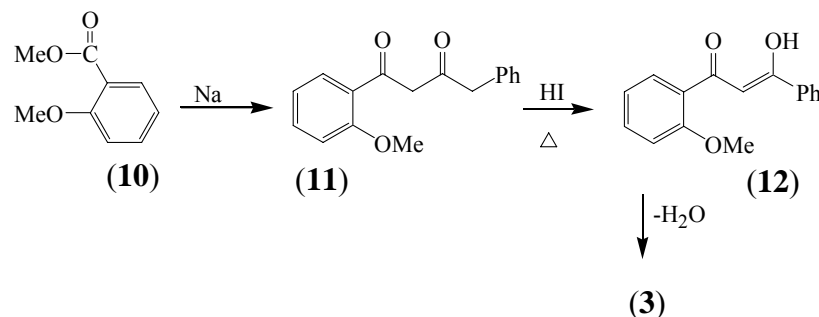
Scheme 1.1 : The Von-Konstanecki method

1.1.2 The Baker-Venkataraman Method

The Baker-Venkataraman approach would be the most convenient route to the synthesis of flavone as shown in **Scheme 1.2**. In Baker-Venkataraman reaction, 2-hydroxyacetophenone (24) was converted to ester (25), which then underwent rearrangement by intramolecular Claisen condensation in the presence of potassium hydroxide and pyridine to afford 1,3-diketone (26). Compound (26) was then cyclised to flavone (3) under rather harsh conditions for either by treatment with concentrated sulfuric acid or heating with glacial acetic acid [15].
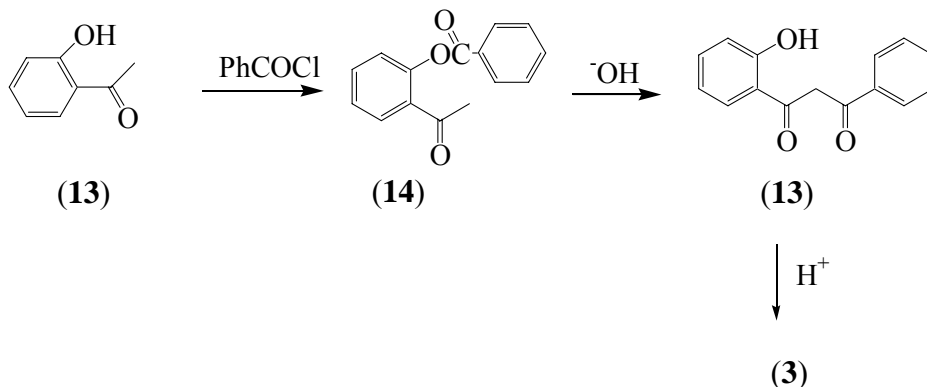
Scheme 1.2 : The Baker-Venkataraman method

1.1.3 Ganguly's Synthesis of Favone

Over a period of years several groups have investigated and improved upon the experimental conditions of Baker–Venkataraman reaction amongst which the
work of Ganguly and colleagues [30]. Using modified Baker–Venkataraman reaction, a novel class of 3-acylflavones which is the precursors to flavones have been synthesised.

In their procedure, compounds such as 2',4'-dihydroxyacetophenone (**14a**) and 2',4',6'-trihydroxyacetophenone (**14b**) were heated with acyl chloride (**15**) in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and pyridine to obtain 3-acyl flavones (**16a**) and (**16b**). On heating under reflux with an aqueous solution of 5% potassium carbonate, compound (**16a**) and (**16b**) yielded (**17a**) and (**17b**), respectively as shown in **Scheme 1.3**.

(**14a**) $R_1 = OH, R_2 = H$
(**14b**) $R_1 = R_2 = OH$

(**16a**) $R_1 = OH, R_2 = H, R_3 = Ph$
(**16b**) $R_1 = R_2 = OH, R_3 = Ph$
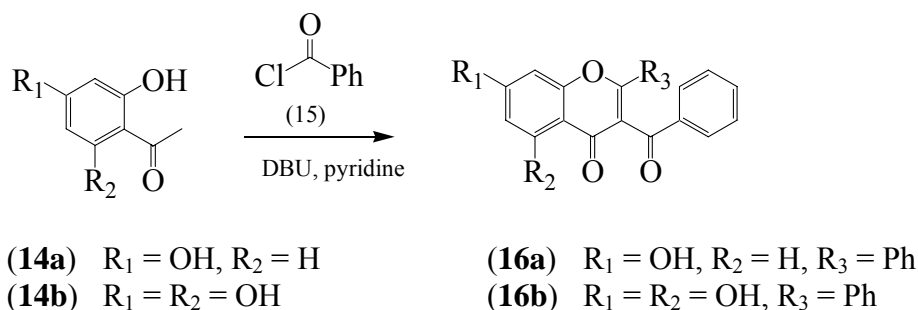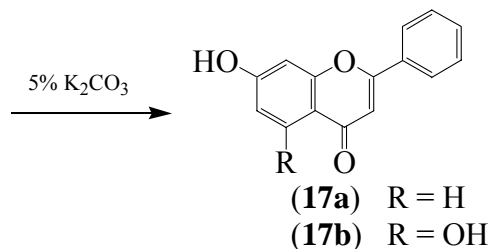
Scheme 1.3 : Ganguly's synthesis of flavone

1.1.4 Synthesis of Calcones via Suzuki Coupling Reaction

Chalcone (**2**) or 1, 3-diphenyl-2-propen-1-one and especially chalcones bearing a hydroxyl function on the aromatic rings are the precursors of all the flavonoids [16]. In 2003, Edrarir and co-workers reported an efficient synthesis of chalcones based on the Suzuki coupling reaction between benzoyl chloride and phenylvinylboronic acid.

Phenylvinylboronic acid was prepared by dehydrogenative borylation of *para*-methoxystyrene (31) by pinacolborane oxidative addition-dehydrogenation catalyzed by the rhodium complex, $RhCl(cod)_2$ to give *p*-methoxyphenylethenylboronic acid pinacol ester (32). This was followed by the oxidative cleavage of (32) using sodium periodate in THF/water to form the *p*-methoxyphenylvinylboronic acid (33) required for the Suzuki coupling step. The coupling between (34) and (33) afforded 3',4',4-trimethoxychalcone (35) under the following condition; solvent: anhydrous toluene; catalyst: tetrakis(triphenylphosphine)palladium(O); base: cesium carbonate as shown in **Scheme 1.4**.
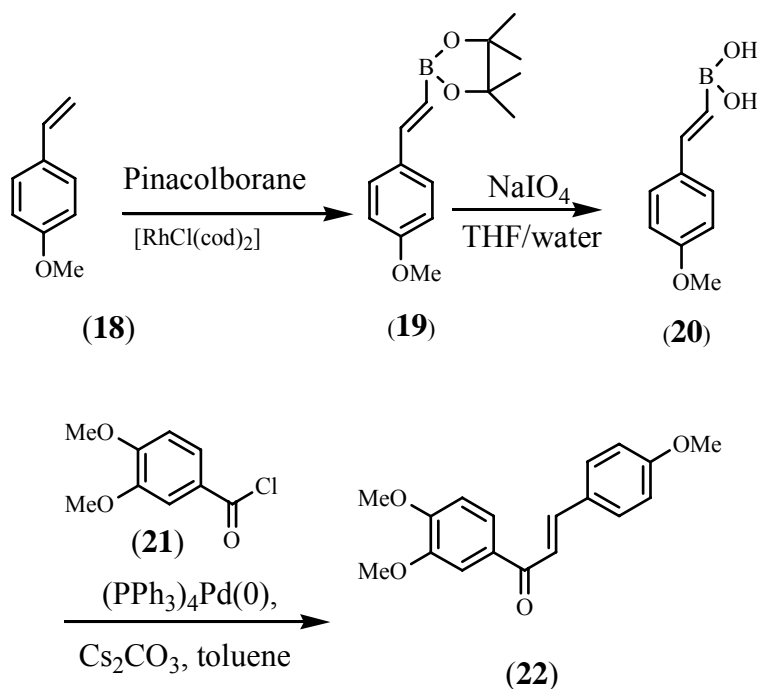
Scheme 1.4 : Synthesis of Calcones via Suzuki Cupling Reaction

1.2 Natural Occurring Polyhydroxyl Flavones

The chemical constituent studies on *Phlomis Nissolii* have yielded luteolin (8) or 5,7,3',4'-tetrahydroxyflavone as well as luteolin-7-rutinoside (**23**) [17]. Luteolin (8), luteolin-4',7-di-*O*-glucoside (**24**) and kaempherol-3-*O*-glucoside (**25**) were isolated from the capitula of *Helichrysum compactum* which have been used as folk medicine for at least 2000 years against gall bladder disorders in the form of medicinal teas [18].

(**23**)
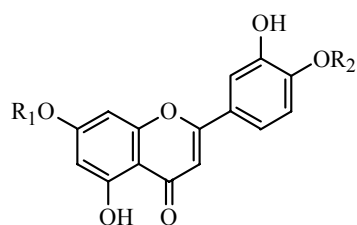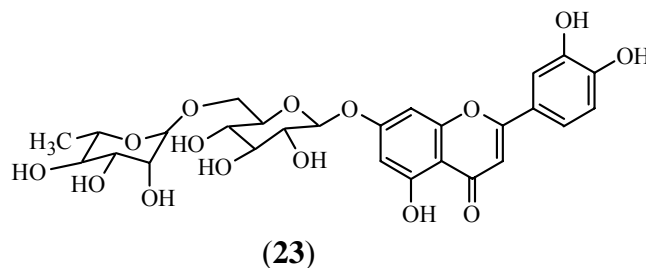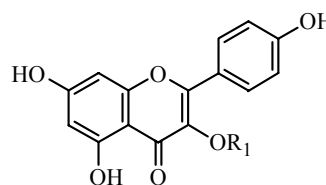
(**24**) R$_1$ = R$_2$ = glu   (**25**)   R$_1$ = glu

Lu *et al.* have isolated few polyphenols from *Salvia officinalis* namely luteolin-3'-*O*-glucuronide (**25**), luteolin 7-*O*-glucuronide (**26**) , 6-hydroxyluteolin 7-*O*-glucoside (**27**), luteolin-7-*O*-glucoside (**28**) and apigenin-6,8-di-*C*-glucoside (**29**). The antioxidant activity of these compounds have been studied and the results obtained showed that those with a catechol B-ring (luteolin glycoside) were more active than compound (**27**) as the presence of the *o*-dihydroxybenzene (catechol) in the B-ring is important to enhance radical-scavenging activities [19].
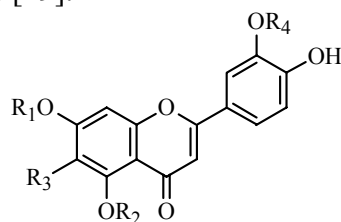
(**26**)   R$_1$ = R$_2$ = R$_3$ = H, R$_4$ = glucu
(**27**)   R$_1$ = glucurose, R$_2$ = R$_3$ = R$_4$ = H
(**28**)   R$_1$ = glucose, R$_2$ = R$_4$ = H, R$_3$ = OH
(**29**)   R$_1$ = glucose, R$_2$ = R$_3$ = R$_4$ = H

1.3　The Research Objective

Research is divided into two parts. Part 1 is focusing on the synthesis of various flavonoids derivatives starting from commercially available starting materials. All the compounds synthesized were characterized by various spectroscopic methods i.e: infrared (IR), ultraviolet (UV), mass spectrometry (MS) and nuclear magnetic resonance (^{1}H and ^{13}C).

Part 2 is on the antioxidant properties and the effect of free radical scavenging activity of the synthesized flavonoids which will be studied by using the Electron Spin Resonance (ESR) spectroscopy method and UV Spectrophotometry method .

CHAPTER II

RESULTS AND DISCUSSION

2.1   Synthesis of 3',4',5,7-Tetramethoxyflavone (28)

The synthetic approach to 3',4',5,7-tetramethoxyflavone (**30**) is as illustrated in **Scheme 2.1**. Our initial step was to prepare the starting material 2-hydroxy-4,6-dimethoxyacetophenone (**32**). Compound (**32**) was derived from commercially available 2,4,6-trihydroxyacetophenone (**31**) by methylation method as shown in **Scheme 1.1**. Compound (**31**) was methylated by using methyl iodide and freshly ignited potassium carbonate in dry acetone. 2-Hydroxy-4,6-dimethoxyacetophenone (**32**) was isolated as colorless crystalline in 35.0% yield with m.p 75-78 °C (lit. [20] 80-81 °C). The IR spectrum of compound (**32**) (Appendix 1) displayed bands characteristics for a hydroxyl group at 3101 cm^{-1} and carbonyl bond at 1618 cm^{-1}.The methylation of (**31**) occurred via the nucleophilic substitution, S$_N$2 reaction of the phenoxide ion and methyl iodide.
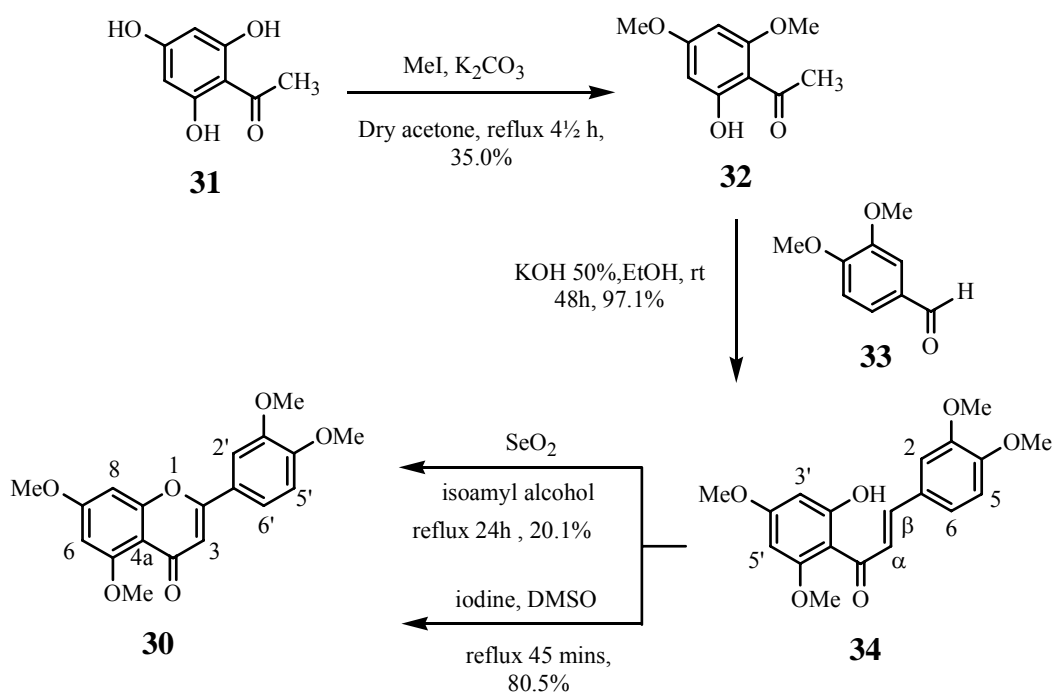
Scheme 2.1 : Synthesis of 3',4',5,7-tetramethoxyflavone (**30**)

The ^{1}H NMR spectrum of (**32**) (Appendix 2) showed two singlets at δ 3.83 (3H) and δ 3.87 (3H) due to the two methoxyl groups at C-4 and C-6 respectively. The

phenolic proton signal appeared as a sharp singlet at δ 14.05. The peaks correspond to the rest of protons appeared at their expected chemical shift values, which was further confirmed by its ^{13}C NMR spectrum (Appendix 3) which displayed nine signals attributing to ten different carbons. The signals for both methoxyl group carbons were overlapping with each other at δ 55.5. Analysis on the DEPT ^{13}C NMR spectrum (Appendix 4) displayed signals for three methyl carbons, five quaternary carbons and two methylene carbons.

The Claisen-Schmidt condensation of 2-hydroxy-4,6-dimethoxyacetophenone (**32**) with commercially available 3,4-dimethoxybenzaldehyde (**33**) under basic condition (KOH 50%) in ethanol proceeded smoothly to furnish 3,4,4',6'-tetramethoxychalcone (**34**) in excellent yield, 97.1%. 3,4,4',6'-Tetramethoxychalcone (**33**) was obtained as yellow-needle crystals, m.p 135-138 °C (lit. [21] m.p 143-145 °C), it was prepared previously [21] as orange yellow solid and on one occasion it was isolated from *Merrillia caloxylon* (Rutaceae) [22]. The structure of chalcone (**33**) was confirmed by spectral data. Chalcone (**33**) has the IR absorptions (Appendix 5) characteristics of carbonyl (1622 cm^{-1}), aromatic C=C (1584 and 1444 cm^{-1}) and C-O (1219 cm^{-1}) functionalities. It gives brown color with alcoholic ferric chloride solution but no IR absorption band for hydroxyl (-OH) group at C-2 position. However, the presence of hydroxyl group at C-2 position was further supported by an appropriately deshielded phenolic proton signal at δ 14.42 (1H, s) in its ^{1}H NMR spectrum (Appendices 6 and 7).

The ^{1}H NMR spectrum of 3,4,4',6'-tetramethoxychalcone (**33**) displayed multiplet due to overlapping of signals for four methoxyl groups at δ 3.88 integrated for 12 protons. The *meta* coupled protons of the A-ring appeared at δ 5.98 (1H, d, *J*= 2.4 Hz, H-5') and δ 6.13 (1H, d, *J*= 2.4 Hz, H-3'). The three aromatic protons of the B-ring were observed at δ 6.92 (1H, d, *J*= 8.2 Hz), δ 7.15 (1H, d, *J*= 1.8 Hz) and δ 7.24 (1H, dd, *J*= 8.2 and 1.8 Hz) assigned to H-5, H-2 and H-6 respectively. The characteristics signals for a chalcone moiety appeared as two doublets at δ 7.79 (1H, d, *J*= 15.6 Hz, H-α) and δ 7.82 (1H, d, *J*= 15.6 Hz, H-β).

The ^{13}C NMR spectrum of chalcone (**33**) (Appendix 8) showed the presence of 16 signals attributed to 19 different carbons. The signals for methyl carbons were overlapping with each other at δ 55.5. The DEPT ^{13}C NMR spectrum of (**33**) (Appendix 9) confirmed the presence of four methyl carbons, eight quaternary carbons and seven methine carbons in this compound. The MS spectrum of (**33**) (Appendix 10) showed a molecular ion peak at *m/z* 344 which was in agreement with the molecular formula $C_{19}H_{20}O_6$.

The final sequence in the synthesis of 3',4',5,7-tetramethoxyflavone (**30**) required the cyclisation of chalcone (**33**) into 3',4',5,7-tetramethoxyflavone (**30**). Cyclisation of chalcone (**33**) into the corresponding flavone (**30**) was carried out differently by using freshly sublimed selenium dioxide in dry isoamyl alcohol and iodine in DMSO. Among the methods used for oxidation of chalcone (83), the iodine in DMSO method gave the highest yield (80.5%) compared to SeO$_2$ method (20.1%). 3',4',5,7-Tetramethoxyflavone (**30**) was isolated as white solid and melted at 187-188°C (lit. [23] 190-191°C).

The UV spectrum (Appendix 11) of 3',4',5,7-tetramethoxyflavone (**30**) displayed absorption bands at 275 and 331 nm suggested the presence of a flavone skeleton [24]. Its IR spectrum (Appendix 12) showed absorption frequencies at 1651 cm^{-1} as well as 1606 and 1510 cm^{-1} indicating the presence of a conjugated carbonyl group and aromatic rings

respectively. The EIMS spectrum (Appendix 13) of flavone (**30**) gave a molecular ion peak at *m/z* 342, which was in agreement with the molecular formula $C_{19}H_{18}O_6$. In the ^{1}H NMR spectrum (Appendix 14), the absence of a phenolic proton signal at downfield region was noted, indicating that the oxidative cyclisation of chalcone (**33**) has took place. This is also supported by the presence of a characteristic singlet signal for a flavone at δ 6.63 (1H, s, H-3). The aromatic protons of the B-ring gave an AMX coupling system at δ 6.98 (1H, d, *J*= 8.4 Hz, H-5'), 7.34 (1H, d, *J*= 2.1 Hz, H-2') and 7.53 (1H, dd, *J*= 2.4 and 8.4 Hz, H-6'). Whereas the two aromatic protons of the A-ring appeared as an AX system at δ 6.40 (1H, d, *J*= 2.1 Hz, H-6) and 6.58 (1H, d, *J*= 2.1 Hz, H-8).

Structure of 3',4',5,7-tetramethoxyflavone (**30**) was further supported by [1]H-^{1}H correlations found in the 2D COSY spectrum (Appendix 15), which showed the following series of connectivities: the signal of H-8 (δ 6.40, d) was coupled with H-6 (δ 6.58, d); and H-6' (δ 7.53, dd) was coupled with H-5' (δ 6.98, d) and H-2' (δ 7.34, d). The ^{13}C NMR spectrum (Appendix 6 and 17) displayed the presence of seven quaternary carbons, six methine carbons and four methyl carbons. The spectroscopic properties of synthetic (**30**) is comparable to those of the natural product isolated recently from *Ottonia corcovadensis* [25]. At this point, 3',4',5,7-tetramethoxyflavone (**30**) was synthesized in 27.4% overall yield.

2.2   Synthesis of 3',4',5,7-Tetrahydroxyflavanone (**39**).

In continuation of our ongoing research in the area of antioxidant compounds, we have synthesized 3',4',5,7-tetrahydroxyflavanone (**39**). The synthesis of 3',4',5,7-tetrahydroxyflavanone (**39**) was accomplished starting from 2,4,6-trihydroxyacetophenone (**31**) as shown in **Scheme 1.2**. To prepare the desired compounds in high yields, the hydroxyl groups in the starting acetophenone were protected as the MOM-ether derivatives in a step-wise manner with methoxymethyl (MOM) chloride. Regioselective methoxymethylation of the 2- and 4-hydroxyl groups was achieved with the use of the strong base dimethylaminopyridine (DMAP) to give the bis-MOM ether, 2-hydroxy-4,6-bis(methoxymethyloxy)-acetophenone (**35**). Compound (**35**) was achieved as brown liquid.

The ^{1}H NMR spectrum (Appendix 19) of compound (**35**) showed the presence of two methoxyl groups at δ 3.47 and 3.53 as two singlets integrating for three protons each. The singlet signals for the methylene protons were observed at δ 5.19 and 5.27. The three methyl protons adjacent to carbonyl group (-COCH_3) appeared at δ 2.67 integrating for three protons. The C-5 and and C-3 protons appeared as doublets at δ 6.26 and 6.28 integrating for one proton each, respectively. The IR spectrum of (**35**) (Appendix 18) displayed stretching bands for hydroxyl group at 3443 cm^{-1} and carbonyl bond at 1623 cm^{-1} The above spectral data suggested that compound (**35**) would be 2-hydroxy-4,6-bis(methoxymethyloxy)-acetophenone. Similarly, the hydroxyl groups of the 3,4-dihydroxybenzaldehyde (**36**) was also protected in an analogous manner as above to yield 3,4-bis(methoxymethyloxy)benzaldehyde (**37**) which was obtained as a brown liquid in 75.1% yield. Its IR spectrum (Appendix 20) showed bands for carbonyl at 1690 cm^{-1}, aromatic rings at 1595 and 1444 cm^{-1} and C-O function at 1262 cm^{-1}. The ^{1}H NMR spectrum (Appendix 21) of 3,4-bis(methoxymethyloxy)-benzaldehyde (**35**) showed the presence of two methoxyl groups at δ 3.53 integrating for six protons. The signal for

methylene protons were observed at δ 5.31 and 5.34. The C-5, C-6, and C-2 protons appeared as doublets at δ 7.29, 7.52 and 7.69 integrating for one proton each, respectively.The signal for the aldehyde proton appeared as a sharp singlet at δ 9.87. The total of 10 peaks corresponding to 11 different carbons were observed in the ^{13}C NMR spectrum of compound (**35**) (Appendices 22 and 23).
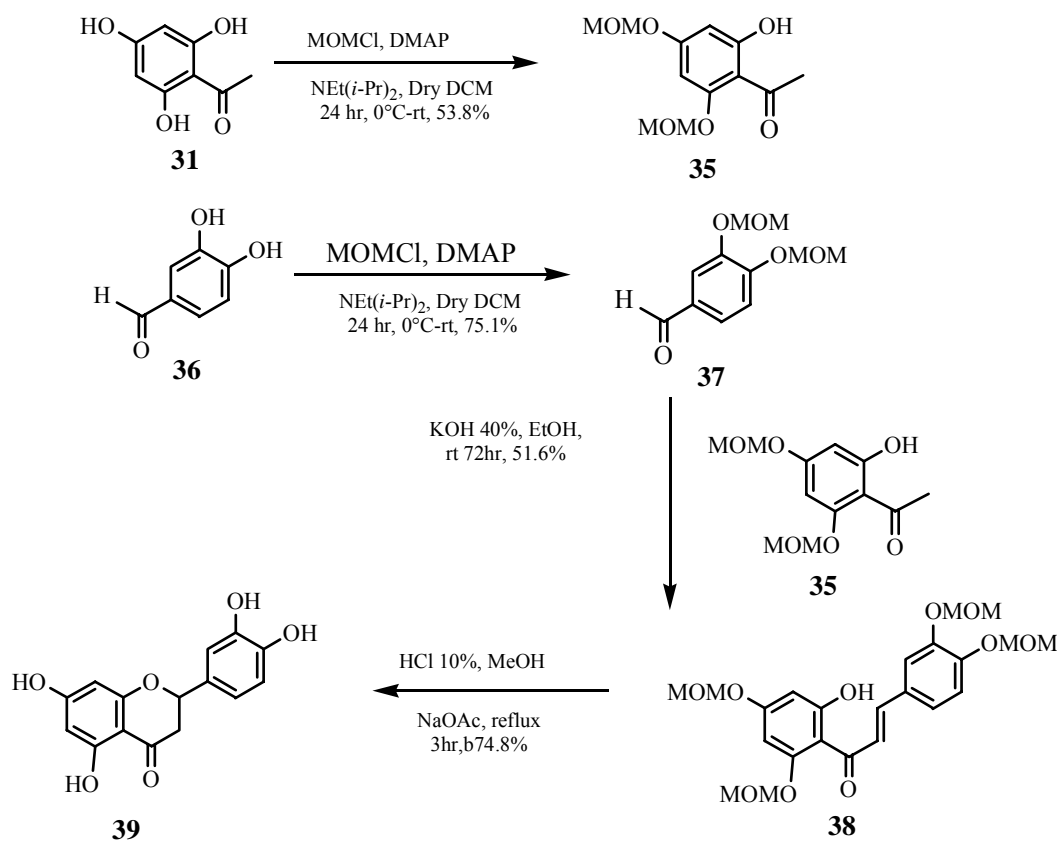
Scheme 2.2 : Route to synthesis of 3',4',5,7-tetrahydroxyflavanone (**39**)

Alkaline condensation of 2-hydroxy-4,6-bis(methoxymethyloxy)-acetophenone (**35**) and 3,4-bis(methoxymethyloxy)-benzaldehyde (**37**) produced 3,4,4',6'-tetrakis(methoxymethyloxy)-chalcone (**38**) in 51.6 % as yellow crystals, m.p 88-90°C (ref. [26] m.p 91-92°C). Chalcone (**38**) gives brown color with alcoholic ferric chloride solution but no IR (Appendix 24) absorption band for hydroxyl group (-OH) at C-2 position. This is supported by (i) a relatively weak IR band at 1625 cm^{-1} (chelated >C=O) and an appropriately deshielded phenolic proton signal at δ 13.9 (1H, s). In the ^{1}H NMR spectrum (Appendix 25), the methylene protons of the protecting group appear as multiplets at δ 5.25 integrating for eight protons, the three aromatic protons of the B ring, which appeared at δ 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) integrating for one proton each. The two aromatic protons of the A ring appeared as two doublets at δ 6.29 (1H, d, *J*= 2.2 Hz), 6.33 (1H, d, *J*= 2.2 Hz) assigned to C-3' and C-5' protons respectively. The ^{1}H NMR also exhibited the presence of a set of *trans*-olefinic protons at δ 7.56 (*J*= 15.6 Hz) and 7.88 (*J*= 15.6 Hz) integrating for one proton each assigned to C$_\alpha$-H and C$_\beta$-H. The ^{13}C NMR spectrum of (**38**) (Appendices 26

and 27) showed the presence of 21 peaks attributed to 23 different carbons in the molecule. The signals for four methyl groups were seen at δ 56.0 and overlapped with each other.

The deprotection of the –MOM group was achieved by hydrolysis of the MOM-protecting group with dilute HCl in methanol and subsequently, treatment with excessive solid sodium acetate afforded us 3',4',5,7-tetrahydroxyflavanone or eriodictyol (**39**) as a single product in high yield, 74.8% with m.p. 194-196 °C (ref. [27] 196-197 °C). It had two UV (Appendix 28) absorption maxima at 331 (sh) and 288 nm indicated the presence of a flavanone skeleton [24]. Compound (**39**), obtained as a brown solid showed characteristic signals for a flavanone at δ 2.67 (1H, dd, *J*= 17.1 and 3.0 Hz, H-3), 3.19 (1H, dd, *J*= 17.1 and 12.6 Hz, H-3) and 5.37 (1H, dd, *J*= 12.5 and 3.3 Hz, H-2), in its ^{1}H NMR spectrum (Appendix 29). The carbonyl group at C-4 was hydrogen bonded with C-5 OH group, as evidenced by a signal which appeared as a singlet at δ 12.13 in its ^{1}H NMR spectrum. The singlet signals for three other phenolic protons were observed at δ 9.04 (1H, H-3'), 9.09 (1H, H-4') and 10.80 (1H, H-7) integrating for one proton each. The aromatic protons of the A ring were observed at δ 5.87 (s, 2H, 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 presence of cross peaks in its COSY (^{1}H-^{1}H) spectrum (Appendix 30) confirmed the distinctive couplings between H-2, which is attached to the chiral centre, and the adjacent protons, H-3a and H-3b. The ^{13}C NMR spectrum (Apppendix 31 and 32) of eriodictyol (**27**) showed 15 carbons. The spectroscopic data of 3',4',5,7-tetrahydroxyflavanone was in good agreement with those of synthetic (**39**) as recently published by Selenski [28].

2.3   Antioxidant studies on the synthesized compounds

2.3.1   Electron Spin Resonance (ESR) Spectrometry Method

In this study, the antioxidative activities of the synthesized flavonoids were measured in terms of its' radical-scavenging ability. The method employed was by determining the free radical inhibitory ability of different antioxidant by using very stable free radicals such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) in ethanolic solution. In the ESR technique, the direct scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (**64**) radical by compound (**34**), (**30**), (**38**) and (**39**) were determined. The DPPH radical has a deep purple color, whereas the reduction product DPPH$_2$ is yellowish. An ESR signal is directly proportional to the number of radicals present. The 0.25 mM DPPH radicals give typical ESR spectrum as shown in **Figure 2.1**. The peaks height will be reduced when an antioxidant was added to the ethanolic DPPH solution. The radical scavenging activity of flavonoids was expressed by means of IC$_{50}$ which represent the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%. **Figure 2.1** showed the intensities of the DPPH signal at concentration of 15.63 µg/mL for the active flavonoids and vitamin C as positive control.

(a)
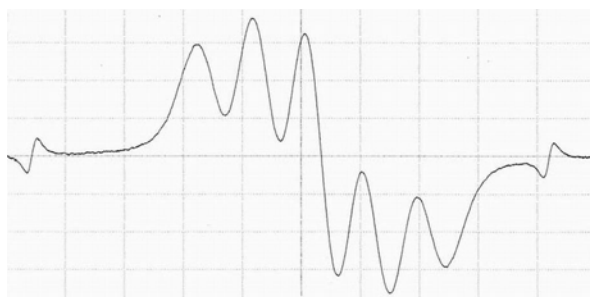
(b)
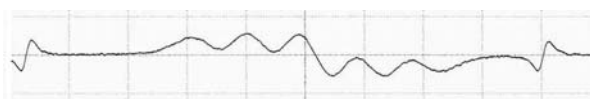
(c)
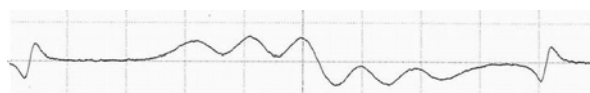
Figure 2.11 : ESR spectra of scavenging effects of (b) eriodictyol (**27**) (percent inhibition: 83.11 and (c) vitamin C (percent inhibition: 80.19%) against (a) DPPH (0.25 mM) free radical.

The radical scavenging activity of the active compounds was found to be concentration-dependent manner. The data reveal that 3',4',5,7-tetrahydroxyflavanone (**39**) possess the strongest activity as free radical scavenger compared with positive control, vitamin C and the other flavonoids, which can be seen from the IC_{50} value of this compound; 8.57 µg/mL. All the chalcones tested showed low radical scavenging activity with IC_{50} more than 100 µg/mL.

2.3.2 UV Spectrophotometry Method

The radical scavenging activity was determined from the reduction in the optical absorbance at 517 due to scavenging of stable free radical of DPPH. Vitamin C was used as a reference antioxidant. In its radical form, DPPH$^\bullet$ absorbs at 515 nm, but upon reduction by an antioxidant or a radical species ($R^\bullet$), the absorption disappears. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and the presence of this free radical makes the solution purple.

The variation of the IC_{50} values between the radical scavenging activity of the compounds by UV spectrophotometer and ESR spectrometry were likely due to the difference concentration of DPPH radical used in the two different experiments. Nevertheless, the results correlated well with the results obtained from the ESR spectrometry assay.

CHAPTER III

METHODOLOGY

3.0 General

Dry acetone was prepared by drying over calcium chloride while methylene chloride (CH_2Cl_2) was obtained by drying over phosphorus pentoxide(P_2O_5) and isoamyl alcohol was obtained by drying over potassium carbonate (K_2CO_3). Petroleum ether refers to boiling range 60-80 °C and was redistilled before use. Reagents were used without further purification except for selenium dioxide which was purified by sublimation. Reactions were monitored by thin-layer chromatography (tlc) carried out on 0.2 mm Merck *pre-coated* silica gel plates (60 F_{254}) and compounds were visualized with UV light. Column chromatography was carried out using Merck silica gel 60 (0.063-0.200 mm). Mass spectral data were obtained from Department of Chemistry, Universiti Malaya. ^{1}H and ^{13}C NMR spectra (300 and 75 MHz respectively) were recorded on a Bruker Avance 300 Spectrometer using deuterated chloroform, $CDCl_3$ as solvent. Infrared (IR) spectra were recorded on a Shimadzu 8000 or Perkin-Elmer series 1600 spectrometers as thin film (NaCl windows) for liquid samples or KBr pellet for solid samples. Melting points were measured on a Leica Galen III micro melting point apparatus and were uncorrected. Antioxidant test was carried out by using the ESR technique. The ESR spectra were recorded on a JEOL 100 JES-FA series spectrometer using manganese oxide (MnO) as an internal standard.

3.1 Synthesis of 3',4',5,7-Tetramethoxyflavone (30)

2-Hydroxy-4,6-dimethoxyacetophenone (32). A well-stirred mixture of 2,4,6-trihydroxyacetophenone (**31**) (1.5 g, 8.93 mmol), methyl iodide (3.16g, 22.32 mmol) and anhydrous potassium carbonate (8 g) in dry acetone was refluxed for 4 ½ hour. The reaction was monitored by tlc. The reaction mixture was filtered and evaporated under reduced pressure. The residue was purified by column chromatography (Pet. ether-EtOAc, 9:1) to give **2-hydroxy-4,6-dimethoxyacetophenone (32)** in (0.61 g, 35.0%) as colourless crystals, m.p 75-78 °C (lit. [4] m.p 80-81°C) and R_f 0.64 (PE: EtOAc, 3: 2); IR ν_{max} (KBr) cm^{-1}: 3101 (O-H), 1618 (C=O), 1424 (C=C aromatic); NMR δ_H ($CDCl_3$) ppm: 2.63 (3H, s, -COCH$_3$), 3.83 (3H, s, -OCH$_3$), 3.87 (3H, s, -OCH$_3$), 5.94 (1H, d, *J*= 2.0 Hz, H-5), 6.08 (1H, d, *J*= 2.0 Hz, H-3), 14.05 (1H, s, -OH); NMR δ_C ($CDCl_3$) ppm: 106.0 (C-1, C-4°), 163.0 (C-2, C-4°), 93.0 (C-3, C-H), 167.5 (C-4, C-4°), 91.0 (C-5, C-H), 166.0 (C-6, C-4°), 55.5 (-OCH$_3$, CH$_3$), 33.0 (-COCH$_3$) and 203.0 (C=O, C-4°).

3',4',4,6-Tetramethoxychalcone **(34).**To a solution of 2-hydroxy-4,6-dimethoxyacetophenone (**93**) (200 mg, 1.11 mmole) in ethanol (15 mL) was added 3,4-dimethoxybenzaldehyde (**94**) (276 mg, 1.66 mmole) followed by addition of KOH (50 %) (2.0 mL). The reaction mixture was left at room temperature for 48h. After that, the reaction mixture was poured into iced water and was acidified with HCl (10%). The reaction mixture was then poured into water and extracted with dichloromethane (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. The resulting syrup was chromatographed on a silica gel column (9: 1 pet-ether- EtOAc) to give **3',4',4,6-tetramethoxychalcone (34)** (371 mg, 97.1%) as yellow crystals, m.p 135-138 °C (lit. [5] m.p 143-145 °C) and R_f 0.28 (PE: EtOAc, 3: 2); IR ν_{max} (KBr) cm^{-1}: 1622 (C=O), 1584 and 1444 (C=C aromatic), 1219 (C-O); NMR δ_H (CDCl$_3$) ppm: 3.88 (12H, m, -OCH$_3$), 5.98 (1H, d, J= 2.4 Hz, H-5'), 6.13 (1H, d, J= 2.4 Hz, H-3'), 6.92 (1H, d, J= 8.2 Hz, H-5), 7.15 (1H, d, J= 1.8 Hz, H-2), 7.24 (1H, d, J= 8.2 and 1.8 Hz, H-6), 7.79 (1H, d, J= 15.6 Hz, H-α), 7.82 (1H, d, J= 15.6 Hz, H-β), 14.4 (1H, s, -OH); NMR δ_C (CDCl$_3$) ppm: 55.5 (-OCH$_3$, CH$_3$), 91.5 (C-5', C-H), 94.0 (C-3', C-H), 107.5 (C-1', C-4°), 110.5 (C-2, C-H), 112.0 (C-5, C-H), 122.5 (C-6, C-H), 126.0 (C-α, C-H), 129.0 (C-1, C-4°), 142.5 (C-β, C-H), 149.0 (C-4, C-4°), 151.5 (C-3, C-4°), 162.5 (C-2', C-4°), 166.0 (C-6', C-4°), 168.0 (C-4', C-4°) and 192.5 (C=O, C-4°); MS: m/z 344 [M$^+$, C$_{19}$H$_{20}$O$_6$, (71)], 151 (100) and 164 (56).

3',4',5,7-Tetramethoxyflavone (30). 3',4',4,6-Tetramethoxychalcone (**34**) (40 mg, 0.12 mmol) was added to dry isoamyl alcohol (3 mL). The mixture was heated in an oil bath so that the entire compound was completely dissolved in the solvent. This was followed by the addition of sublimed SeO$_2$ (39 mg, 0.35 mmole). The reaction mixture was refluxed for 24 hours. The reaction mixture was then filtered and dried in vacuum. A tlc examination showed it to be a mixture of two components. The mixture was purified by column chromatography on a silica gel column with (Pet. ether: EtOAc, 2: 3) as eluent. The isolated compound was recrystallized with PE to afford **3',4',5,7-tetramethoxyflavone (30)** (10 mg, 20.1%) as white solid, m.p 187-188°C (lit. [8] 190-191°C) and R_f 0.13 (PE; EtOAc, 2: 3). It gives blue fluorescence in UV light; IR ν_{max} (KBr) cm^{-1}: 1651 (C=O), 1606 and 1510 (C=C aromatic), 1263 (C-O); UV λ_{max} (MeOH) nm: 275, 331; NMR δ_H (CDCl$_3$) ppm: 3.98 (12H, m, -OCH$_3$), 6.40 (1H, d, J= 2.4 Hz, H-2'), 6.58 (1H, d, J= 2.1 Hz, H-6), 6.63 (1H, s, H-3), 6.98 (1H, d, J= 8.4 Hz, H-5'), 7.34 (1H, d, J= 2.1 Hz, H-8), 7.53 (1H, dd, J= 2.4 and 8.4 Hz, H-6'); NMR δ_C (CDCl$_3$) ppm: 55.0-56.5 (-OCH$_3$, CH$_3$), 93.0 (H-6, C-H), 96.5 (C-3, C-H), 108.0 (C-8, C-H), 109.5 (C-2', C-H), 111.5 (C-5', C-H), 119.5 (C-6', C-H), 124.5 (C-1, C-4°), 150 (C-4', C-4°), 152.0 (C-3', C-4°), 159.5 (C-8a, C-4°), 160.5 (C-5, C-4°), 161.5 (C-2, C-4°) and 164.0 (C-7, C-4°); EIMS: m/z 342 [M$^+$, C$_{19}$H$_{18}$O$_6$, (100)], 341 (51), 325 (11), 313 (24) and 296 (29).

Synthesis of 3',4',5,7-tetramethoxyflavone (30) using iodine in DMSO. A solution of compound (**34**) (50 mg, 0.145 mmol) and iodine (1.84 mg, 0.0073 mmole) in DMSO (3mL) was refluxed for 45 minutes. The mixture was poured into water, and the resulting syrup was extracted with EtOAc (2 x 15 mL). The organic phase was then washed with sodium thiosulphate solution, water and brine. The organic layer was dried over

anhydrous MgSO$_4$ and the solvent was then evaporated under reduced pressure. The residual syrup was chromatographed on a silica gel column with (PE: EtOAc, 2: 3) as eluent to afford **3',4',5,7-tetramethoxyflavone (30)** (40 mg, 80.5%) as yellow crystals. It gives blue fluorescence under UV light. Spectral data of flavone (**30**) were also similar to that of the product prepared by the selenium dioxide, SeO$_2$ method.

3.2   Synthesis of 3',4',5,7-Tetrahydroxyflavanone (39)

2-Hydroxy-4,6-bis(methoxymethyloxy)-acetophenone (35). To a suspended solution of 2,4,6-trihydroxyacetophenone (**31**) (0.5 g, 2.976 mmole) in CH$_2$Cl$_2$ (10 mL) at 0 °C, *N,N*-diisopropylethylamine (6 mL) was added, this was followed by the addition of DMAP (35.2 mg, 0.29 mmole) and the reaction mixture was stirred for 15 mins. MOMCl (175 mg, 2.17 mmole) was then added dropwise 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 chloroform (2 x 20 mL). The chloroform extracts were 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(methoxymethyloxy)-acetophenone (107)** (0.41 g, 53.8%) as a colorless 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, -COCH$_3$), 3.47 (3H, s, -OCH$_3$), 3.53 (3H, s, -OCH$_3$), 5.19 (2H, s, -OCH$_2$), 5.27 (2H, s, -OCH$_2$-), 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); NMR δ$_C$ (CDCl$_3$) ppm: 32.5 (-COCH$_3$), 56.0 (-OCH$_3$), 56.5 (-OCH$_3$), 94.0 (C-5, C-H), 94.5 (-OCH$_2$), 97.5 (C-3, C-H), 107.0 (C-1, C-4°), 160.5 (C-2, C-4°), 163.5 (C-6, C-4°), 167.0 (C-4, C-4°) and 203.0 (C=O, C-4°).

3,4-Bis(methoxymethyloxy)benzaldehyde (37). To a suspended solution of 3,4-dihydroxybenzaldehyde (**36**) (100 mg, 0.724 mmol) in CH$_2$Cl$_2$ (10 mL) at 0°C, *N,N*-diisopropylethylamine (3 mL) was added, this was followed by the addition of DMAP (17.6 mg, 0.145 mmole) and the reaction mixture was stirred for 15 mins. MOMCl (175 mg, 2.17 mmole) was then added dropwise 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 chloroform (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 (9: 1, Pet.ether- EtOAc) to give **3,4-bis(methoxymethyloxy)benzaldehyde (37)** (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, -OCH$_3$), 5.31 (2H, s, -OCH$_2$), 5.34 (2H, s, -OCH$_2$-), 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); NMR δ$_C$ (CDCl$_3$) ppm: 56.0 (-OCH$_3$), 95.0 (-OCH$_2$), 95.5 (-OCH$_2$), 115.0 (C-5, C-H), 115.5 (C-2, C-H), 126.0 (C-6, C-H), 131.0 (C-1, C-4°), 147.5 (C-3, C-4°), 152.5 (C-4, C-4°) and 191.0 (C=O, C-4°).

3,4,4',6'-Tetrakis(methoxymethyloxy)chalcone (38). To a solution of 2-hydroxy-4,6-bis(methoxymethyloxy)acetophenone (**35**) (111 mg, 43.44 mmole) in ethanol (5 mL) was added 3,4-bis(methoxymethyloxy)benzaldehyde (**37**) (108 mg, 47.79 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 iced water and acidified with HCl (10%). The reaction mixture was then poured into water and extracted with dichloromethane (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. The resulting syrup was chromatographed on a silica gel column (PE: EtOAc, 9: 1) to give **3',4',4,6-tetrakis(methoxymethyloxy)chalcone (38)** (104 mg, 51.6%) as yellow crystals, m.p. 88-90 °C (lit [17] m.p. 91-92 °C) and R_f 0.25 (PE: EtOAc, 3: 2); IR ν_{max} (KBr) cm^{-1}: 1581 and 1353 (C=C olefinic), 1625 (C=O); NMR δ_H (CDCl$_3$) ppm: 3.55 (12H, m, -OCH$_3$), 5.25 (8H, m, -OCH$_2$), 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); NMR δ_C (CDCl$_3$) ppm: 56.0 (4-OCH$_3$), 94.5 (C-5', C-H), 97.5 (C-3', C-H), 116.0 (C-2, C-H), 108.0 (C-1', C-4°), 117.0 (C-5, C-H), 124.0 (C-6, C-H), 126.5 (C-α, C-H), 131.5 (C-1, C-4°), 142.0 (C-β, C-H), 148.0 (C-4, C-4°), 149.5 (C-3, C-4°), 160.0 (C-2', C-4°), 163.5 (C-6', C-4°), 167.5 (C-4', C-4°) and 193.0 (C=O, C-4°); MS: m/z 464 [M$^+$, C$_{23}$H$_{28}$O$_{10}$, (10)], 179 (100) and 254 (73).

3',4',5,7-Tetrahydroxyflavanone (39). To 3',4',4,6-tetrakis(methoxymethyloxy)-chalcone (**38**) (0.05 g, 0.108 mmol) in MeOH (6mL) was added HCl (1mL) and the mixture was refluxed for 1.5 h. Then NaOAc (0.18 g, 2.15 mmol) was added and then the resulting mixture was refluxed for 3 h. The mixture was cooled and H$_2$O (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 (39)** in 74.8% yield as a brown solid with m.p 194-196 °C (lit. [18] 196-197 °C) and R_f 0.27 (PE: EtOAc, 1: 4); IR ν_{max} (KBr) cm^{-1}: 3411 (OH), 1655.0 (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$_3$BO$_3$; 323 (sh), 288; NMR δ_H (DMSO) ppm: 2.67 (1H, dd, J= 17.1 and 3.0 Hz, H-3), 3.19 (1H, dd, J= 17.1 and 12.6 Hz, H-3), 5.37 (1H, dd, J= 12.45 and 3.3 Hz, H-2), 5.87 (2H, s, H-6 and H-8), 6.73 (2H, s, H-5' and H-6'), 9.09 (1H, s, -OH), 9.04 (1H, s, -OH), 10.80 (1H, s, -OH), 12.13 (1H, s, -OH); NMR δ_C (DMSO) ppm: 42.5 (C-3, C-H), 79.0 (C-2, C-H), 95.0 (C-6, C-H), 96.0 (C-8, C-H), 102.0 (C-4a,C- C-4°), 114.5 (C-2', C-H), 116.0 (C-5', C-H), 118.0 (C-6', C-H), 130.0 (C-1', C-4°), 145.5 (C-4', C-4°), 146.0 (C-3', C-4°), 163.0 (C-5, C-4°), 164 (C-8a, C-4°), 167.0 (C-7, C-4°), 196.5 (C=O, C-4°); MS: m/z 287 [M-1, C$_{15}$H$_{12}$O$_6$, (7)], 285 (10), 239 (8), 149 (34), 141 (50), 135 (40), 97 (35), 87 (44), 71 (61) and 57 (100)

3.3   Scavenging Action on DPPH Radical (ESR)

A test solution was prepared by preparing 125-1000 μg/mL concentration of compound (46) and ascorbic acid (65) in ethanol. The test solution (200 μL) was mixed with 200 μL of 1000 μg/mL DPPH ethanolic solution in a test tube by shaking for 10 s. The mixture was transferred to a flat cell for analysis of the amount of DPPH radical.

ESR spectra were recorded after 30 s of mixing the solutions. The signal intensity was evaluated by dividing the peak height of the third of the five line signals of the DPPH radical with the height of the MnO signal to give relative peak height. The conditions of the ESR spectrometer were set at power; 0.998 mW, magnetic field; 336.000 ± 5 mT, field modulation width; 0.05 mT, sweep time; 30 s and time constant; 0.03 s. The scavenging effect of DPPH was calculated by following formula:

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

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

The IC_{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.

Table 3.1 : Results of antioxidant for flavonoids by ESR spectrometry method

Sample	Percentage Inhibition Concentration (µg/mL)			
	500	**125**	**31.25**	**15.63**
(34)	60.3±1.8	49.5±0.6	37.3±1.2	32.4±2.3
(30)	NA	NA	NA	NA
(38)	53.77±2.1	41.5±2.4	36.36±1.2	32.6±0.9
(39)	96.22±1.0	94.81±1.1	87.97±1.1	83.11±2.4

Data represent mean ± SD of three independent experiments performed in triplicate
NA= not active

3.4 UV Spectrophotometry Method

A test solution was prepared by preparing 125-1000 µg/mL concentration of compound (46) and ascorbic acid (65) in methanol. When DPPH reacts with an antoxidant compound, which can donate hydrogen, it is reduced. The colour changes (from deep violet to light yellow) were measured at 517 nm. The difference in absorbance between the test sample and control was expressed as percent inhibition was taken as the activity. The percentage inhibition was calculated by the following formula:

$$\text{Scavenging (\%)} = \frac{\text{Abs DPPH- Abs sample}}{\text{Abs DPPH}} \times 100$$

Table 3.2 : Results of antioxidant for flavonoids by UV spectrophotometry method

Sample	Percentage Inhibition Concentration (µg/mL)			
	500	**125**	**31.25**	**15.63**
(34)	57.12±1.1	43.22±0.7	38.1±0.1	38.4±0.8
(30)	NA	NA	NA	NA
(38)	50.18±1.2	38.2±2.1	32.12±0.3	24.32±0.9
(39)	80.12±1.1	73.41±4.1	57.93±2.1	47.21±1.3

Data represent mean ± SD of three independent experiments performed in triplicate

CHAPTER IV

CONCLUSION

4.1 Conclusion

In the present study, the synthesis of a methylated luteolin, namely 3',4',5,7-tetramethoxyflavone (**30**) has been carried out successfully from the starting material 2,4,6-trihydroxyacetophenone (**31**). The methylation of 2,4,6-trihydroxyacetophenone (**31**) formed 2-hydroxy-4,6-dimethoxyacetophenone (**32**) followed by aldol condensation with 3,4-dimethoxybenzaldehyde (**33**) under basic condition formed 3,4,4',6'-tetramethoxychalcone (**34**). Compound (**34**) later underwent oxidative cyclisation to form 3',4',5,7-tetramethoxyflavone (**30**). It was found that the oxidative cyclisation of the chalcone (**34**) using iodine in DMSO gave excellent yield in a short duration compared to with SeO_2.

The protection of 2,4,6-trihydroxyacetophenone (**31**) and 3,4-dihydroxybenzaldehyde (**36**) with methoxy methyl chloride (MOMCl) to form 2-hydroxy-4,6-bis (methoxymethyloxy)-acetophenone (**35**) and 3,4-bis (methoxymethyloxy)-benzaldehyde (**37**) was carried out. Compound (**35**) and (**37**) later underwent the aldol condensation in order to form 3,4,4',6'-tetra (methoxymethyloxy)-chalcone (**38**). Acid hydrolysis (HCl 10% in MeOH) of chalcone (**38**) and subsequently, treatment with excessive sodium acetate afforded us 3',4',5,7-tetrahydroxyflavanone or eriodictyol (**39**) as a single product in high yield.

The antioxidative properties of all synthesized flavonoids were tested by using the ESR and UV spectrophotometry technique. In the ESR technique, the direct scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical by the compounds were determined. The reduction of the ESR signal for DPPH radical after the addition of 3',4',5,7-tetrahydroxyflavanone (**39**) showed that compound (**39**) is a strong antioxidant agent with IC_{50} value, 8.57 µg/mL.

REFERENCES

1. Joule, J.A. and Smith, G.F. Heterocyclic Chemistry. London: Van Nostrand Reinhold Company. 1972.

2. Middleton, E., Kandaswami, C. The Impact of Plant Flavonoids on Mammalia Biology: Implication for Immunity, Inflammation and Cancer. In: Harbone, J.B. The Flavonoids: Advances in Research since 1986. 3rd. Ed. Chapman and Hall. 1994.

3. Geissman, T.A. and Crout, D. H. G. Organic Chemistry of Secondary Plant Metabolism. California: Freeman, Cooper and Company. 1969.

4. Proestos, C., Boziaris I.S. and Nychas, J.E. Analysis of Flavonoids and Phenolic Acids in Greek Aromatic Plants: Investigation of Their Antioxidant Capacity and Antimicrobial Activity. *Food Chem.* 2005. 93: 1998-2004.

5. Yenjai, C., Prasanphen, K., Daodee, S. Kittakoop, P. Bioactive flavonoids from Kaempherilia Parviflora. *Fitoterapia*. 2004. 75: 89-92.

6. Furuta, T., Kimura, T., Kondo, S., Wakimoto, T., Nukaya, H., Tsuji, K. and Tanaka, K. Concise Total Synthesis of Flavone C-glycoside having Potent Anti-inflammatory Activity. *Tetrahedron*. 2004. 60: 9375-9379.

7. Xia, Y., Yang, Z. Y., Xia, P., Bastow, K. F., Nakanishi, Y. and Lee, K.H. Antitumor Agents. Part 202: Novel 2'-Amino Chalcones: Design, Synthesis And Biological Evaluation. *Bioorganic and Medicinal Chemistry Letters*. 2000. 10: 699-701.

8. Hertog, M. G. L. Dietary Antioxidant Flavonoids and Risk of Coronary Heart Disease. The Zuthpen Elderly Study. *Lancet*. 1993. 342: 1007-1011.

9. Mellou, F., Lazari, D., Skaltsa, H., Tselepis, A. D., Kolisis, F. N., Stamats, H. Biocatalytic Preparation of Acylated Derivatives of Flavonoids Glycosides Enhances Their Antioxidant and Antimicrobial Activity. *Journal of Biotechnology*. 2005. 116: 295-304.

10. Rusak, G., and Gutzeit, H.O. Structurally Related Flavonoids with Antioxidative Properties Differentially Effect Cell Cycle Progressive and Apoptosis of Human Acute Leukimia Cells. *Nutrient Research*. 2005. 25: 141-153.

11.   Zhao, F.P., Strack, D., Baumert, A., Subramaniam, R., Ngah, K. G., Tett, F. C., Swee, N. T, Lian, S. C. Antioxidant Flavonoids from Leaves of *Polyganum Hydropiper L.. Phytochemistry*. 2003. 62: 219-228.

12.   Rice Evans, C. A., Miller, N. J., Paganga, G., Antioxidant Properties of Phenolic Compounds. *Trends in Plants. Science Reviews*. April 1997. 2: 152-159.

13.   Wright, J. S., Johnson, E. R., Dilabio, G. A. Predicting The Activity of Phenolic Antioxidants. Theoritical, Method, Analysis of Substituent Effect and Application to Major Families of Antioxidants. *J. Am. Chem. Soc*. 2001. 123: 1173-1183.

14.   Nakanishi, K. Natural Products Chemistry. Vol 2. Tokyo: Kodansha Ltd. 225-228.

15.   Ganguly, A. K., Kaur, S., Mahata, P. K., Biswas, D., Pramanik, B. N. and Chan T. M. Synthesis and Properties of 3-acyl-γ-pyrones, A Novel Class of Flavones and Chromones. *Tetrahedron Letters*. 2005. 46: 4119- 4121.

16.   Edrarir, S., Cotelle, N., Bakkour, Y. and Rolando, C. An Efficient Synthesis of Chalcones Based on the Suzuki Reaction. *Tetrahedron Letters*. 2003. 44: 5359- 5363.

17.   Bucar, F., Ninov, S., Ionkova, I., Kartnig, T., Asenov, I. and Konuklugil, B. Flavonoids from *Phlomis nissolii. Phytochemistry*. 1998. 48 (3): 573-575.

18.   Süzgeç, S., Meriçli, A. H., Houghton, P. J. and Cubukçu. Flavonoids of *Helichrysum compactum* and Their Antioxidant and Antibacterial Activity. *Fitoterapi*a. 2005. 76: 269-272.

19.   Lu, Y. and Foo, L. Y. Antioxidant Activities of Polyphenols from sage (*Salvia officianalis*)*. Food Chemistry*. 2001. 75: 197-202.

20.   Srivastava, S. D., Srivastava, S. K. Synthesis of a New Flavone. *Indian J. Chem*. 1987. **26B**. 57-58.

21.   Chu, H. W., Wu, H.T, Lee, Y. J. Regioselective hydroxylation of 2'-hydroxchalcones by dimethyldioxirane towards polymethoxylated flavonoids. *Tetrahedron*. 2004. **60.** 2647-2655.

22.   Fraser, A. W., Lewis, J. R. Flavonoids from *Merrillia caloxylon. Phytochemistry*. 1974. **13**. 1561-1564.

23.   Banerji, A., Goomer, N. C. A New Synthesis of Flavones*. Synthesis*. 1980. **11**. 874-875.

24.   K. R. Markham, Techniques of Flavonoid Identification, 1982, Academic Press, London.

25.   Facundo, V. A., Morais, S. M. and Filho, R. B. Chemical Constituents of *Ottonia corcovadensis* Miq. from Amazon Forest – [1]H and [13]C Chemical Shift Assignments. *Quim. Nova.* 2004. **27**: 79-83.

26.   Hu, M., Krausz, K., Chen, J., Jun, Ge, X., Li, J., Gelboin, H. Identification of CYP1A2 as the main isoform for the phase I hydroxylated metabolism of genistein and a prodrug converting enzyme of methylated isoflavone. *Drug Metabolism and Disposition*. 2003. **31**(7). 924-931.

27.   Chun, Y., Shi, J.-G., Mo, S.-Y. Chemical constituents of *Pyrrosia petiolosa*. *J. Asian Nat. Prod. Res*. 2003. **5**(2). 143-150.

28.   Selenski, C., Pettus, T. R. R., (±)-Diinsininone: made nature's way. *Tetrahedron*. 2006. **62**. 5298-5307.