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# Synthesis and antioxidant activities of naturally occurring alpinum isoflavone, 4′-O-methylalpinum isoflavone and their synthetic analogues

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**ABSTRACT**

*Naturally occurring isoflavone viz, alpinum isoflavone, 4′-O-methylalpinum isoflavone, and a series of five analogues were synthesized by prenylation of isoflavone* ***(4)*** *which was synthesized from phloroglucinol. The structures of all the synthesized compounds were confirmed using* $^{1}$*HNMR,* $^{13}$*CNMR spectral data and mass spectroscopy. Antioxidant activities of the above compounds were investigated. The synthesized compounds exhibited less significant activity compared to vitamine C.*

**Key words:** alpinum isoflavone, 4′-O-methylalpinum isoflavone, isoflavone, antioxidant, DPPH

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## INTRODUCTION

Isoflavonoids represent class of naturally occurring compounds which exist in the plant kingdom, the families of liguminosae are the major source of these compounds [1], significant amount is found in soybeans [2] and exhibit many important biological activities such as antimicrobial [3], antifungal, antibacterial [4-6], antitumor [7], anticancer [8-10], anti oxidant [11] activities.

Prenylated isoflavonoids mainly distributed in Leguminosae and Moraceae [12] they have been isolated from various plants [13-18]. Prenylated isoflavonoids have increased lipophilicity compared to non prenylated isoflavonoid, which result in enhanced biological activities and exhibit significant pharmacological effect. Studies show that prenylated isoflavonoids have antibacterial, anti fungal [19], anticancer effects as well as in diabetes mellitus treatment [20].

Epidemiological and experimental studies revealed that the consumption of diets, rich in fruit and vegetables lower risks for chronic diseases, such as cardiovascular diseases, arthritis, chronic inflammation and cancers [21]. These diseases thought to be the result of oxidative stress, which is caused by an insufficient capacity of biological systems to neutralize excessive free radical production [22]. Antioxidants protect the body from damage caused by free radicals. They exert their effect by scavenging the free radicals (i.e. reactive oxygen species (ROS) or reactive nitrogen species) universally present in biological systems [23].

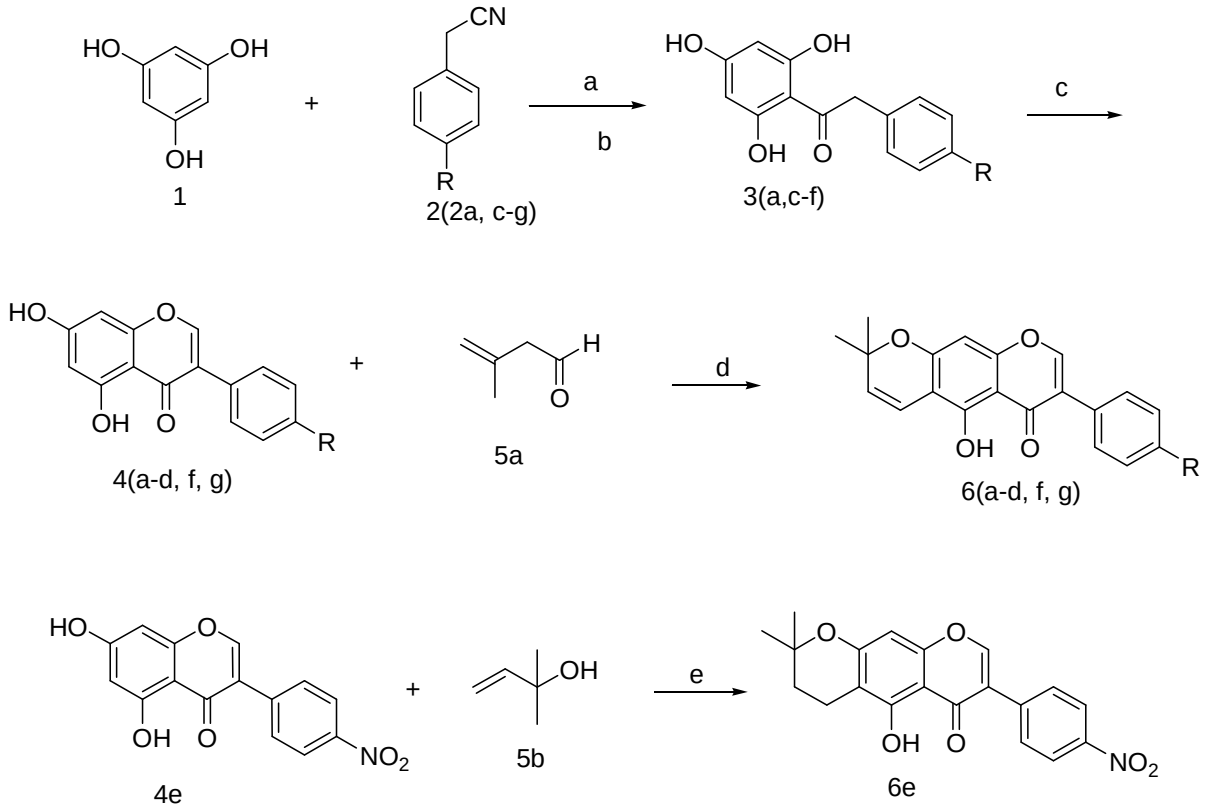
## MATERIALS AND METHODS

### 1.1. General

All chemicals and solvents were purchased from Sigma Aldrich and used without further purification; the reaction process was monitored by TLC silica gel plates, the purification of the products was performed using column chromatography using silica gel (100-200 mesh), Melting points were measured in open capillary tubes, and were uncorrected. Infrared (IR) spectra were recorded using FT-IR Bruker Alpha spectrometer. NMR spectra were recorded on Bruker (400 MHz) spectrometer and Jeol JNM EX-90 NMR spectrometer using TMS as the internal standard, mass spectra were recorded on an Agilent 110 Lc/MSD. The antioxidant activity of all the target compounds **6(a-g)** was investigated using DPPH (1, 1 diphenyl-2-picrylhydrazyl) radical method.

### 1.2. Chemistry

The titled compounds **6** (**a-g)** described in this study were synthesized as outlined in **Scheme 1.** Acylation of phloroglucinol with appropriately substituted phenyl acetonitrile catalyzed by HCl gas and $ZnCl_2$, in dry ether afforded imines, which was further hydrolyzed to the intermediate, substituted 2'4'-6'-trihydroxydeoxybenzoins, **3(a, c-g**). Cyclization of the intermediate 3(**a, c-g)** on treatment with $Et_2O.BF_3$, DMF/$POCl_3$ yielded substituted isoflavonoids 4**(a, c-f)** in good yield. Compound **4b** was synthesized by demethylation of **4a** using anhydrous aluminium trichloride. The target compounds **6**(**a-d & f, g)** were synthesized by direct prenylation of **4(a-d, & f, g)** with 3-methylbut-3-enal (**5a**) in the presence of $Ca(OH)_2$, and compound **6e was** synthesized by prenylation of **4e** with 2-methyl-3-buten-2-ol (**5b**) in DCM in the presence of PPA, in each case mixture of products were obtained but only the major product was crystallized and characterized by advanced spectroscopic data, the spectral characterization were presented bellow;




**R: a. OMe, b. OH, c. H, d. F, f. $CH_3$, g . Br**

**Scheme 1: Reagent and condition: a) HCl $_{(gas)}$, $ZnCl_2$, $Et_2O$, 0 $^oC$ b) $H_2O$, reflux c) DMF, $POCl_3$, 60-70$^0$C, 4hrs d) Ca $(OH)_2$, MeOH, RT, 36 hrs, e) PPA, DCM**

**1.3. Synthesis and characterization.**

**1.3.1. General procedure for the Synthesis of substituted 2', 4', 6'-trihydroxy deoxybenzoins 3(a, c-g)**

Dry HCl gas was passed through the ice cold solution of (5 g, 0.039 mol) of phloroglucinol, **(1)** (0.044 mol) of substituted phenylacetonitrile, **2 (a, c-g)**, and 2 g of anhydrous zinc chloride in 100 ml of anhydrous ether for 2 h stirring continuously. The mixture was allowed to stand in refrigerator overnight and again hydrogen chloride gas was passed through the mixture for another 2 hours. The mixture was allowed to stand in refrigerator for three days. The ether was decanted and washed twice with ether; the solid was hydrolyzed by refluxing with 100 ml of 2 % HCl water for 2 hour. On cooling, filtering with suction filtration and drying in oven afforded the target compounds.

**1-(2, 4, 6-Trihydroxy)-2-(4-methoxyphenyl)ethanone (3a)**

Yield: 52%; Mp: 190-194 $^{o}$C; Anal. Calc. (%) for $C_{15}H_{14}O_5$: C, 65.69; H, 5.15; O, 29.17; found: C, 65.62; H, 5.19; O, 29.19; $^{1}$H NMR (DMSO-$d_6$, 400 MHz): δ 12.94 (s, 2 H, -OH), 10.92 (s, 1H, -OH), 7.2 (d, $J$ = 8.0 Hz, 2H, Ar-H), 6.8 (d, $J$ = 8.0 Hz, 2H, Ar-H), 5.8 (s, 2H, Ar-H), 4.2 (s, 2H), 3.8 (s, 3H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz): δ 202.69, 164.78, 164.20, 157.84, 130.58, 127.75, 113.54, 103.63, 94.70, 54.97, 47.97; ESI-MS: *m/z* 275 $[M + H]^+$.

**1-(2,4,6-Trihydroxyphenyl)-2-phenylethanone (3c)**

Yield: 47%, Mp: 158-161 $^{o}$C; Anal. Calc. (%) for $C_{14}H_{12}O_4$: C, 68.85; H, 4.95; O, 26.20; found: C, 68.81; H, 4.98; O, 26.21; $^{1}$H NMR (DMSO-$d_6$, 400 MHz,): δ 12.22 (s, 2H, -OH), 10.39 (s, 1H, -OH), 7.31-7.19 (m, 5H, Ar-H), 5.83 (s, 2H, Ar-H), 4.35 (s, 2H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz): δ 202.2, 164.8, 164.2, 135.9, 129.6, 128.0, 126.1, 103.7, 94.7, 48.8; ESI-MS: *m/z* 245 $[M + H]^+$.

**2-(4-Fluorophenyl)-1-(2,4,6-trihydroxyphenyl)ethanone (3d)**

Yield: 40 %, Mp: 194-196 $^{o}$C; Anal. Calc. (%) for $C_{14}H_{11}FO_4$: C, 64.12; H, 4.23; O, 24.40; found: C, 64.07; H, 4.25; O, 24.43; $^{1}$H NMR (DMSO-$d_6$, 400 MHz,): δ 12.19 (s, 2H, -OH), 10.39 (s, 1H, -OH), 7.27-7.23 (m, 2H, Ar-H), 7.11 (t, $J$ = 9.2, 2H, Ar-H), 5.83 (s, 2H, Ar-H), 4.34 (s, 2H); $^{13}$CNMR (DMSO-$d_6$,100 MHz): δ 202.0, 164.8, 164.2, 160.9 (d, $^{1}J_{CF}$ = 241 Hz, 1C), 132.0(d, $^{4}J_{CF}$ = 3 Hz, 1C), 131.4 (d, $^{3}J_{CF}$ = 8.0 Hz, 2C), 114.6 (d, $^{2}J_{CF}$ = 21 Hz, 2C, ) 103.6, 94.7, 48.0; ESI-MS: *m/z* 263 $[M + H]^+$.

**1-(2,4,6-Trihydroxyphenyl)-2-(4-nitrophenyl)ethanone (3e)**

Yield: 58 %, Mp: 222-224 $^{o}$C; Anal. Calc. (%) for $C_{14}H_{11}NO_6$: C, 5.13; H, 3.83; N, 4.84; O, 33.19; found: C, 5.09; H, 3.84; N, 4.85; O, 33.21; $^{1}$H NMR (DMSO-$d_6$, 400 MHz): δ 12.21 (s, 2H, -OH), 10.92 (s, 1H, -OH), 8.22 (d, $J$ = 8.8 Hz, 2H, Ar-H), 7.55 (d, $J$ = 8.8 Hz, 2H, Ar-H), 5.89 (s, 2H, Ar-H), 4.57 (s, 2H,); $^{13}$C NMR (DMSO-$d_6$, 100 MHz): $\delta$ = 200.8, 165.1, 164.2, 146.2, 144.2, 131.1, 123.0, 103.7, 94.7, 48.83; ESI-MS: *m/z* 290 $[M + H]^+$.

**1-(2,4,6-Trihydroxyphenyl)-2-p-tolylethanone (3f)**

Yield: 56%, Mp: 168-170 $^{o}$C; Anal. Calc. (%) for $C_{15}H_{14}O_4$: C, 69.76; H, 5.46; O, 24.78; found: C, 69.72; H, 5.48; O, 24.80; $^{1}$H NMR (DMSO-$d_6$, 400 MHz): δ 12.22 (s, 2H, -OH), 10.38 (s, 1H, -OH), 7.09 (s, 4H, Ar-H), 5.81 (s, 2H, Ar-H), 4.28 (s, 2H,), 3.01(s, 3H, $CH_3$); $^{13}$C NMR (DMSO-$d_6$, 100 MHz): δ 202.5, 164.8, 164.2, 135.1, 132.8, 129.4, 128.6, 103.6, 94.6, 48.4, 20.6; ESI-MS: *m/z* 259 $[M + H]^+$.

**2-(4-Bromophenyl)-1-(2,4,6-trihydroxyphenyl)ethanone (3g)**

Yield: 48%, Mp: 218-220 °C; Anal. Calc. (%) for $C_{14}H_{11}BrO_4$: C, 52.04; H, 3.43; Br, 24.73; O, 19.80; found: C, 52.01; H, 3.44; O, 19.82; $^{1}$H NMR (DMSO-$d_6$, 400 MHz):δ 12.12 (s, 2 H, -OH), 10.41 (s, 1 H, -OH), 7.45 (d, $J$ = 8.4 Hz, 2H, Ar-H), 7.18 (d, $J$ = 8.8 Hz, 2H, Ar-H), 5.82 (s, 2 H, Ar-H), 4.33 (s, 2H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz): δ 201.6, 164.9, 164.2, 135.3, 131.9, 130.8, 119.4, 103.6, 94.7, 48.31; ESI-MS: *m/z* 323 $[M + H]^+$.

**General procedure for cyclization of substituted 2', 4', 6'-trihydroxydeoxybenzoins (Synthesis of (4a, 4c-g))**

With cooling and vigorous stirring (3 mL, 24 mmol) of etherated boron triflouride was added drop wise to (8 mmol) of (**3a, 3c-g**) in 3ml of DMF. The cooling was stopped and (0.9ml. 9.6 mmol) of phosphrous oxychloride was added drop wise, after mixing all the components the reaction mixture was stirred at 60-70 $^{o}$C for 2 h and then poured into acidified water the precipitate was filtered off and purified by column chromatography using hexane : ethylacetate (8:2) as eluents.

**5, 7-dihydroxy-3-(4-methoxyphenyl)-*4H*-chromen-4-one (4a)**

Yield: 61%, Mp: 212-214 $^{o}$C; Anal. Calc. (%) for $C_{16}H_{12}O_5$: C, 67.60; H, 4.25; O, 28.14; found: C, 67.57; H, 4.27; O, 28.15; $^{1}$H NMR (DMSO-$d_6$, 400 MHz): δ 12.94 (s, 1H, 5-OH), 10.92 (s, 1H, 7-OH), 8.38 (s, 1H, 2-H), 7.50 (d, $J$

= 8.0 Hz, 2H, Ar-H), 7.0 (d, *J* = 8.0 Hz, 2H, Ar-H), 6.40 (1H, Ar-H), 5.77 (s, 1H, Ar-H), 3.79 (s, 3H); $^{13}$CNMR (DMSO-$d_6$, 100 MHz,): δ 180.0, 164.3, 161.9, 159.1, 157.5, 154.2, 130.1, 122.9, 121.9, 113.7, 104.4, 99.0, 93.6, 55.1; ESI-MS: *m/z* 285 [M + H]$^+$; FT-IR (KBr, Cm$^{-1}$): 3312 (O-H), 3007, (C-H aromatic), 2950 (C-Haliphatic), 1642 (C=O).

**Demethylation of (4a) (preparation of 5, 7-dihydroxy-3-(4-hydroxyphenyl)-4*H*-chromen-4-one) (4b)**

Aluminum chloride was heated to 140 $^o$C for about 45 min. and then **4a** suspended in toluene was added, heating continued for 6 h, after completion of the reaction, the reaction mixture was cooled and added to HCl solution to break the $AlCl_3$ complex, the solution was filtered and dried washed with toluene.

Yield: 84 %, Mp: 292-294 $^o$C; Anal. Calc. (%) for $C_{15}H_{10}O_5$: C, 66.67; H, 3.73; O, 29.60; found: C, 66.62; H, 3.76; O, 29.62; $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 12.94 (s, 1 H, 5-OH), 10.9 (s, 1H, 7-OH), 9.61 (s, 1H, 4′-OH), 8.31 (s, 1H, 2-H), 7.38 (d, *J* = 4.0 Hz, 2 H, Ar-H), 6.82 (d, *J* = 4.0 Hz, 2H, Ar-H), 6.39 (s,1H, Ar-H); $^{13}$C NMR (DMSO-$d_6$,100 MHz): δ 180.2, 164.2, 161.9, 157.5, 157.4, 153.9, 130.1, 122.2, 121.2, 115.0, 104.4, 98.9, 93.6; ESI-MS: *m/z* 271 [M + H] $^+$.; FT-IR (KBr, Cm$^{-1}$): 3412 (-OH), 3004, (C-H aromatic), 2933(C-H aliphatic), 1652 (C=O).

**5,7-dihydroxy-3-phenyl-*4H*-chromen-4-one (4c)**

Yield: 76%, Mp: 194-196 $^o$C*;* Anal. Calc. (%) for $C_{15}H_{10}O_4$: C, 70.86; H, 3.96; O, 25.17; found: C, 70.82; H, 3.98; O, 25.19; $^1$H NMR (DMSO-$d_6$**,** 400 MHz): δ 10.81 (s, 1H, 7-OH), 8.39 (s, 1H, 2-H), 8.0 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.58 (d, *J* = 8.0 Hz, 2H, Ar-H), 6.97 (d, *J* = 2.0 Hz, 1H, Ar-H), 6.88 (d, *J* = 2 Hz, 1H, Ar-H); $^{13}$C NMR (DMSO-$d_6$, 22.5 MHz): δ 174.3, 162.6, 157.4, 153.6, 153.5, 132.1, 128.8, 127.6, 123.5; ESI-MS: *m/z* 255 [M + H]$^+$; FT-IR (KBr, Cm$^{-1}$): 3198 (O-H), 3062, (C-H aromatic), 2925 (C-Haliphatic), 1626 (C=O).

**3-(4-fluorophenyl)-5,7-dihydroxy-*4H*-chromen-4-one (4d)**

Yield: 54%, Mp: 188–190 °C; Anal. Calc. (%) for $C_{15}H_9FO_4$: C, 66.18; H, 3.33; F, 6.98; O, 23.51 found: C, 66.13; H, 3.34; O, 23.55; $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 12.83 (s, 1H, 5-OH), 10.93 (s,1H, 7-OH), 8.44 (s, 1H, 2-H), 7.63 (t, *J* = 8.8 Hz, 2H, Ar-H), 7.31 (t, *J* = 9.2 Hz, 2H, Ar-H), 6.42 (d, *J* = 1.6 Hz, 1H, Ar-H), 6.25 (d, *J* = 1.6 Hz, 1H, Ar-H); $^{13}$CNMR (DMSO-$d_6$, 22.5 MHz): δ 179.1, 164.6, 161.8, 156.2, 157.3, 147.0, 157.2, 156.2, 147.0, 137.8, 123.1; ESI-MS: m/z 273 [M + H] $^+$; FT-IR (KBr, Cm$^{-1}$): 3299 (O-H), 3077, (C-H aromatic), 2924 (C-H aliphatic), 1664 (C=O).

**5, 7-Dihydroxy-3-(4-nitrophenyl)-*4H*-chroman-4-one (4e)**

Yield: 63%, Mp: 286-288 $^o$C; Anal. Calc. (%) for $C_{15}H_9NO_6$: C, 60.21; H, 3.03; N, 4.68; O, 32.08; found: C, 60.17; H, 3.05; N, 4.69; O, 32.09; $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 12.70(s, 1 H, 5-OH), 11.01 (s, 1H, 7-OH), 8.63 (s, 1H, 2-H), 8.32 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.91(d, *J* = 8.8 Hz, 2H, Ar-H) 6.46 (d, *J* = 1.6 Hz, 1H, Ar-H), 6.28 (d, *J* = 2 Hz, 1H, Ar-H); $^{13}$C NMR (DMSO-$d_6$, 22.5 MHz): δ 179.1, 164.6, 161.9, 157.4, 156.1, 146.8, 137.9, 123.1, 120.3, 104.3, 99.2; ESI-MS: m/z 300 [M + H] $^+$.; FT-IR (KBr, Cm$^{-1}$): 3418 (O-H), 1654 (C=O).

**5, 7-dihydroxy-3-tolyl-*4H*-chromen-4-one (4f)**

Yield: 68%, Mp: 193-195 $^o$C*;* Anal. Calc. (%) for $C_{16}H_{12}O_4$; C, 71.64; H, 4.51; O, 23.86; found: C, 71.61; H, 4.53; O, 23.87; $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 12.90 (s, 1H, 5-OH), 10.89, (s, 1H, 7-OH), 8.38 (s, 1H, 2-H), 7.46 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.26 (d, *J* = 8.0 Hz, 2H, Ar-H), 6.40 (d, *J* = 2.0 Hz, 1H, Ar-H), 6. 24 (d, *J* = 2.0 Hz, 1H, Ar-H), 2.34 (s, 3H,); $^{13}$C NMR (DMSO-$d_6$, 100 MHz**):** δ 179.9, 164.5, 161.9, 157.5, 157.4, 154.5, 137.3, 128.8, 128.7, 127.8, 122.2, 104.4, 99.0, 93.7, 20.7; ESI-MS: *m/z* 269 [M + H]$^+$; FT-IR (KBr, Cm$^{-1}$): 3264 (O-H), 3073, (C-H aromatic), 2921 (C-Haliphatic), 1643 (C=O).

**3-(4-bromophenyl)-5,7-dihydroxy-*4H*-chromen-4-one (4 g)**

Yield: 66%, Mp: 212-214 °C*;* Anal. Calc (%) for $C_{15}H_{14}BrO_5$: C, 54.08; H, 2.72; Br, 23.99; O, 19.21; found: C, 54.06; H, 2.73; O, 19.22; $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 12.79 (s, 1H, 5-OH), 10.94, (s, 1H, 7-OH), 8.47 (s, 1H, 2-H), 7.66 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.55 (d, *J* = 8.4 Hz, 2H, Ar-H), 6.42 (d, *J* = 1.6 Hz, 1H, Ar-H), 6.25 (d, *J* = 1.6 Hz, 1H, Ar-H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz): δ 179.5, 164.5, 161.9, 155.1, 131.1, 130.9, 121.3, 121.1, 99.2, 93.8; ESI-MS: *m/z* 332 [M+H]$^+$.; FT-IR (KBr, Cm$^{-1}$): 3374 (O-H), 3063, (C-H aromatic),2919 (C-Haliphatic), 1657 (C=O).

**General procedure for the synthesis of 6(a-d & f, g)**

A mixture of (7 mmol) of **4** (**a-d, f, g)** and (14 mmol) of $Ca(OH)_2$ was dissolved in methanol and (35mmol) of 3-methylbut-3-enal was added and stirred at room temperature for 36 hrs. Methanol was removed using rotary

evaporator and extracted with ethylacetate, the combined organic layer was washed with 1N HCl three times and purified by column chromatography, eluent (hexane: ethylacetate, 9:1) to give the target compound.

**5-Hydroxy-3-(4'-methoxy) phenyl-8,8-dimethyl-4H;8H-benzo[1,2b;4b]dipyran-4-one (6a)**
Yield: 42%, M.P: 182-186 $^{o}$C; Anal. Calc. (%) for $C_{21}H_{18}O_5$: C, 71.99; H, 5.18; O, 22.83; found: C, 71.95; H, 5.21; O, 22.84; $^{1}$H NMR ($CDCl_3$, 90 MHz): $\delta$ 13.14 (1H, s, H-5-OH), 7.81 (1H,s, H-2), 7.49 (d, *J* = 8.1 Hz, 2H, , H-2' and H-6'), 7.0 (d, *J* = 8.1 Hz ,2H, H-3' and H-5'), 6.78 (d, *J* = 10.8 Hz, 1H, H-4"), 6.32 (s, 1H, H-8), 5.66 (d, *J* = 9.9 Hz, 1H, H-3"), 3.83 (s, 3H, OCH3), 1.47 (s, 6H, 2CH3); **$^{13}$C** NMR ($CDCl_3$, 22.5 MHz): δ 180.8, 159.7, 159.4, 157.2, 156.9, 152.5, 149.8, 130.0, 128.0, 123.4, 123.0, 115.4, 114.0, 106.0, 105.5, 94.8, 94.6, 55.4, 28.3; ESI-MS: *m/z* 351 $[M+H]^+$.

**5-Hydroxy-3-(4′-methoxy) phenyl-8,8-dimethyl-4H;8H-benzo[1,2b;4b]dipyran-4-one (6b)**
Yield: 46%, Mp: 166-168 $^{o}$C; Anal. Calc. (%) for $C_{21}H_{18}O_5$: C, 71.42; H, 4.79; O, 23.78; found: C, 71.37; H, 4.81; O, 23.81; $^{1}$H NMR ($CDCl_3$, 90 MHz): δ 13.08 (1H, s, H-5-OH); 7.73 (1H, s, H-2), 7.42(d, *J* = 9 Hz, 2H, , H-2' and H-6'), 6.94 (d, *J* = 9 Hz ,2H, H-3' and H-5'), 6.7 (d, *J* = 10.8 Hz, 1H, H-4"), 6.24 (s, 1H, H-8), 5.59 (d, *J* = 9.9 Hz, 1H, H-3"), 1.39 (s, 6H, 2CH3); ESI-MS: *m/z* 336 $[M+H]^+$.

**5-Hydroxy-3-phenyl-8,8-dimethyl-4H;8H-benzo[1,2b;4b]dipyran-4-one (6c)**
Yield: 41%, Mp: 138-140 $^{o}$C; Anal. Calc. (%) for $C_{20}H_{16}O_4$: C, 74.99; H, 5.03; O, 19.98; found: C, 74.93; H, 5.06; O, 20.00; $^{1}$H NMR ($CDCl_3$, 90 MHz): δ 7.84 (s, 1H, 2H), 7.47 (5H, aromatic H), 6.79 (d, *J* = 9.9Hz, 1H, H-4"), 6.34(s, 1H, H-8), 5.67 (d, *J* = 9.9 Hz, 1H, H-3"), 1.47 (s, 6H); ESI-MS: *m/z* 321 $[M+H]^+$; FT-IR (KBr, $Cm^{-1}$): 3424.24 (O-H), 2966(C-H aliphatic), 1654(C=O).

**5-Hydroxy-3-(4'-flouro) phenyl-8,8-dimethyl-4H;8H-benzo[1,2b;4b]dipyran-4-one(6d)**
Yield: 35 %, Mp: 130-132 $^{o}$C; Anal. Calc. (%) for $C_{20}H_{15}FO_4$: C, 71.00; H, 4.47; F, 5.62, O, 18.92; found: C, 70.92; H, 4.52; O, 18.94; $^{1}$H NMR ($CDCl_3$, 400 MHz): δ 13.04 (s, 1H, 5-OH), 7.83 (s, 1 H, 2-H), 7.51 (dd, *J* = 8.4, 5.2 Hz, 2 H, Ar-H), 7.15 (t, *J* = 8.4 Hz, 2 H, Ar-H), 6.73 (d, *J* = 10.4, 1H, H-4" ), 6. 34 (s, 1H, Ar-H), 5.64 (d, *J* = 10.4, 1H, H-3"), 1.57 (d, 6H); $^{13}$CNMR ($CDCl_3$, 100 MHz): δ 180.5, 164.1, 159.7, 157.3 (d, $^{1}J_{CF}$ = 39 Hz, 1C), 152.8, 130.7, (d, $^{4}J_{CF}$ = 8 Hz, 1C), 128.3, 126.7, 123.0, 115.7, 115.5, (d, $^{3}J_{CF}$ = 10 Hz, 2C), 106.0, (d, $^{2}J_{CF}$ = 33, 2C), 95.00, 78.20, 28.35; ESI-MS m/z: 339 $[M+H]^+$.

**5-Hydroxy-3-(4′-nitro) phenyl-8, 8-dimethyl-6,7-dihydro-4H;8H-benzo[1,2b;4b]dipyran-4-one (6e)**
To a solution of (4e) (1 equivalent) in DCM, PPA (0.1 equivalent) and 2-methyl-3-buten-2-ol (1 equivalent) was added, the mixture was stirred at rt for 1 h, and then refluxed for 4 h, after completion of the reaction, (monitored by TLC) the reaction mixture was cooled, neutralized with saturated $NaHCO_3$ solution, extracted with ethylacetate, the combined organic layer was dried over sodium sulphate, concentrated under reduced pressure. The crude residue was purified by column chromatography over silica gel.

Yield: 56%, Mp: 198-202 $^{o}$C; Anal. Calc. (%) for $C_{20}H_{17}NO_6$: C, 65.39; H, 4.66; N, 3.81; O, 26.13; found: C, 65.33; H, 4.69; O, 26.16; $^{1}$H NMR ($CDCl_3$, 90 MHz): δ 8.25 (d, *J* = 8.8 Hz, 2 H, Ar-H), 7.87 (s, 1H, 2-H), 7.76 (d, 2 H, *J* = 8.8 Hz, Ar-H), 2.65 (t, *J* = 7.2, 2H), 1.88 (t, *J* = 6.8, 2H), 1.39 (s, 6H); $^{13}$CNMR ($CDCl_3$, 100 MHz): δ 174.5, 156.7, 155.0, 153.5, 147.2, 139.7, 130.1, 124.3, 108.6, 107.0, 99.6, 75.2, 32.6, 17.1, 16.3; ESI-MS: *m/z* 366 $[M+H]^+$.

**5-Hydroxy-3(4′-methyl) phenyl-8,8-dimethyl-4H;8H-benzo[1,2b;4b]dipyran-4-one(6f)**
Yield: 51 %, Mp: 81-83 $^{o}$C; Anal. Calc. for $C_{21}H_{18}O_4$: C, 75.43; H, 5.43; O, 19.14; found: C, 75.36; H, 5.47; O, 19.16; $^{1}$H NMR ($CDCl_3$, 400 MHz): δ 13.16 (s, 1H, 5-OH), 7.83 (s, 1 H, 2-H), 7.41 (d, *J* = 8 Hz, 2 H, Ar-H), 7.26 (d, *J* = 6.4 Hz, 2 H, Ar-H), 6.73 (d, *J* = 10, 1H, H-4" ),

6. 33 (s, 1H, Ar-H), 5.64 (d, *J* = 10, 1H, H-3"), 2.3 (s, 3H), 1.47 (s, 6H); $^{13}$CNMR ($CDCl_3$, 22.5 MHz): δ 180.8, 159.5, 157.3, 157.0, 152.7, 138.3 , 129.3, 128.8, 128.2, 127.8, 123.8, 115.5, 106.1, 105.6, 94.9, 78.0, 28.3, 21.2; ESI-MS: *m/z* 335 $[M+H]^+$; FT-IR (KBr, $Cm^{-1}$): 3441(O-H), 3063 (C-H aromatic), 2975 (C-H aliphatic), 1649 (C=O).

**5-Hydroxy-3(4′-bromo) phenyl-8,8-dimethyl-4H;8H-benzo[1,2b;4b]dipyran-4-one(6g)**
Yield: 40 %, Mp: 86-88 $^{o}$C; Anal. Calc. for $C_{20}H_{15}BrO_4$: C, 60.17; H, 3.79; Br, 20.01; O, 16.03; found: C, 60.11; H, 3.82; O, 16.06; 1H NMR ($CDCl_3$, 400 MHz): δ 12.99 (s, 1H, 5-OH), 7.84

(s, 1H, 2-H), 7.57 (d, *J* = 8 Hz, 2 H, Ar-H), 7.40 (d, *J* = 8 Hz, 2 H, Ar-H), 6.72 (d, *J* = 10, 1H, H-4"), 6. 33 (s, 1H, Ar-H), 5.61(d, *J* = 10, 1H, H-3"), 1.47 (s, 6H); 13CNMR ($CDCl_3$, 100 MHz): δ 180.3, 159.7, 157.2, 156.8, 152.9, 131.7, 130.5, 129.7, 128.3, 122.8, 122.6, 115.4, 106.0, 105.8, 95.0, 78.2, 28.3; ESI-MS: *m/z* 401/399 $[M+H]^+$; (for $^{81}Br/^{79}Br$, 100%, 99%)

## RESULTS AND DISCUSSION

### 1.4. Biological activity

The DPPH free radical scavenging activities of the synthesized compounds (6a-g) were evaluated as detailed bellow. DPPH radical scavenging effect was carried out by using the most widely used DPPH methods [24].

100 μM DPPH (1,1-diphenyl-2-picrylhydrazyl) : 3.943 mg of DPPH was dissolveed in methanol and made up to 100 ml to obtain a final concentration of 100 μM.

Standard and test items preparation: stock of standard (3 mg/ml) and test items (25mg/mL) was prepared in distilled water and DMSO, respectively. Appropriate dilution of standard (25 μg/mL-0.39 μg/mL) and test items (100 μg/mL- 1.5 μg/mL) was prepared.

20 μL of test items and 280 μL of DPPH reagent were added to reach a final volume of 300 μL and incubated in dark for 50 minutes and then absorbance were measured at 517 nm using spectrophotometer.

The radical scavenging activities were expressed as the inhibition percentage and were calculated using the formula:
Percentage inhibition (%) of DPPH = [(A– B / A) ×100]

A – Difference in absorbance of control sample between samples with and without DPPH
B – Difference in absorbance of test sample between sample with and without DPPH
Vitamin C was employed as standard. The $IC_{50}$ value was calculated for each of the synthesized compounds as well as the standard; the result was summarized in Table I and graphically represented in Figure 1:

**Table 1: DPPH radical scavenging activity of the synthesized compounds**

| Entry | Compound code | $IC_{50}$(μg/mL) |
|---|---|---|
| **1** | **6a** | >100 |
| **2** | **6b** | >100 |
| **3** | **6c** | >100 |
| **4** | **6d** | >100 |
| **5** | **6e** | >100 |
| **6** | **6f** | >100 |
| **7** | **6g** | >100 |
| | **standard** | $IC_{50}$ **(μg/mL)** |
| | **Vitamin-C** | 2.63 |
| | **Vitamin-C** | 2.61 |

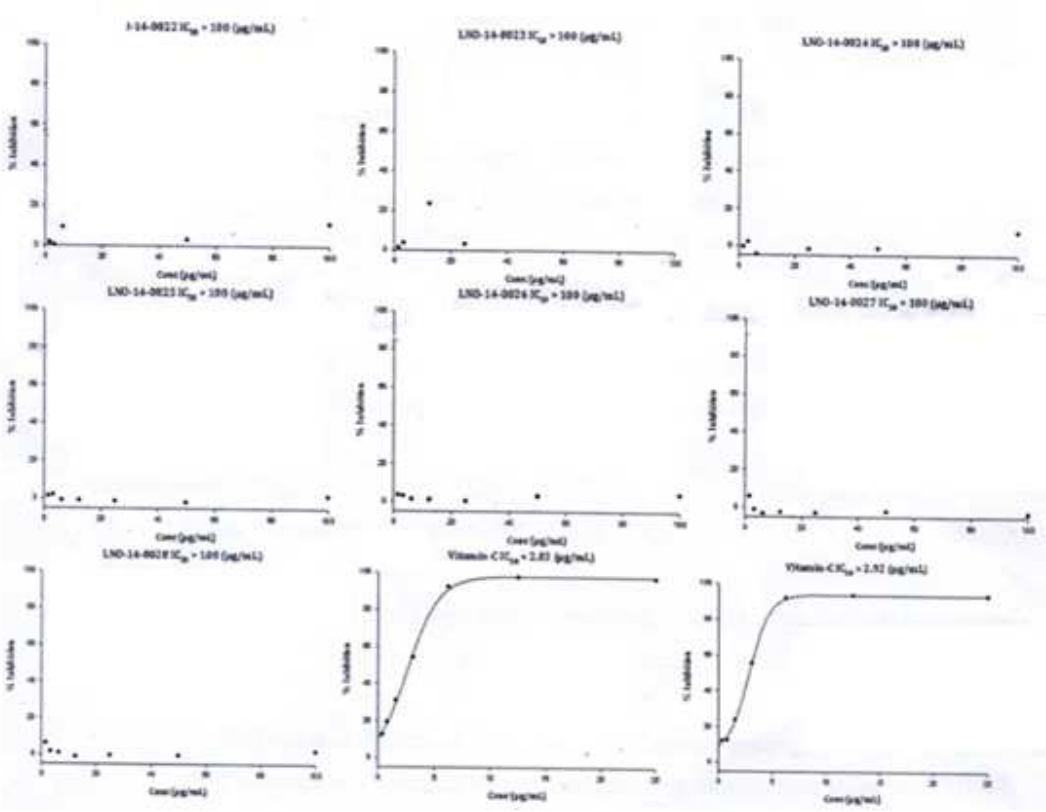


**Figure 1: DPPH radical scavenging activity of the synthesized compounds 6 (a-g) and the standard**

## CONCLUSION

Hydroxyl groups are necessary for isoflavones and flavones to possess antioxidant activity, for some favonoids with similar basic structures the peroxyl radical absorbing capacity is proportional to the number of free hydroxyl groups on their structure [32]. Our studies confirm the above. All the synthesized compounds show no significant activity, this may be because the 7-OH group which contribute to antioxidant activity of isoflavonoids was blocked by prenylation, further more the presence of various substituents on 4′ position of B-ring has little or no effect in the activity of the molecules because $IC_{50}$ value of all the synthesized compounds is greater than (100µg/ml) despite the presence of various substituents.

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