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A Study on Hydrogen Peroxide Scavenging Activity and Ferric Reducing Ability of Simple Coumarins

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Abstract

Coumarin compounds are δ-lactones where α-pyrone ring is fused with benzene ring. Coumarins are widely distributed in the plant kingdom as well as they are very important in synthetic organic chemistry. Coumarins have great interest because of their abundance in nature and diverse pharmacological activities including antibacterial, antiviral, antipyretic, and anticoagulant, antioxidant, anti-inflammatory and anticancer. This study focused on synthesizing different simple Coumarins and studying their antioxidant activity. Four simple Coumarins (Coumarin (C_1), 4-hydroxy coumarin (C_2), 7-hydroxy coumarin (C_3) and 7-hydoxy-4-methyl coumarin (C_4)) were synthesized by using standard methods and were characterized by using UV, IR, ^{1}H and ^{13}C NMR spectra. Antioxidant activity of the simple Coumarins was studied by using standard FRAP assay and Hydrogen peroxide assay and expressed as FRAP value (mmol Fe^{2+}/g) and IC_{50} value (mg/dm^{-3}) respectively. Ascorbic acid was used as standard. All synthesized simple Coumarins showed both antioxidant activities. Hydroxyl Coumarins (C_2, C_3 and C_4) showed higher activities in both cases than C_1. Among the hydroxyl Coumarins C_3 showed highest hydrogen peroxide scavenging activity and Ferric reducing capacity too. Antioxidant power of the tested simple Coumarins in decreasing order was C_3, C_2, C_4 and C_1 in both cases. But the hydrogen peroxide scavenging activity and Ferric reducing capacity of the all synthesized simple Coumarins were lower when compared to standard ascorbic acid.

Keywords:

Coumarin;
Hydroxyl coumarins;
Antioxidant;
Hydrogen peroxide assay;
FRAP assay.

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1- Introduction

Coumarin compounds represent an important class of naturally occurring and synthetic heterocyclic compounds. They have α-benzopyrone framework where, a benzene ring is fused with α-pyrone ring (Figure 1). This frame work allows it to show various properties. As well as, different substitutions in various positions on the frame work influence its properties. Coumarin, which is the parent compound of the benzo-α-pyrone group, owed its name from 'Coumarou', the vernacular name of the Tonka bean (*Dipteryx odorata Wild*), from which Coumarin was first isolated by Vogel of Munich in 1820 [1]. Coumarin was first chemically synthesized by the English chemist, William Henry Perkin In 1868 [2]. Several coumarin derivatives are distributed in the plant kingdom. They are found in more than 40 different plant families, among them Rutaceae and Umbelliferae are the richest sources of coumarins. They are also found at high levels in some essential oils and spices (eg. Cassia cinnamon, sweet clover, peppermint, etc.) [3]. Esculetin, Umbelliferone and Scopoletin are the most widespread coumarins in nature. Coumarins are also isolated from microbial sources (eg. Novobiocin, Coumermycin, Aflatoxins, etc.) [4]. Recent study shows the occurrence of coumarin derivative (Versicolone A) in insect – associated fungus, *Aspergillus versicolor* too [5].The Simple coumarins are the hydroxylated, alkoxylated and alkylated derivatives of the parent compound, Coumarin. Coumarins have immense interest not only for their abundant in nature but also for their diverse biological activities such as anticoagulant, vasorelaxant, antioxidant, antidepressant,

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anticonvulsant, antihistaminic, anticancer, antimicrobial, anti-inflammatory, anti-tumor, antiasthmatic, antituberculosis and antihyperlipidemic activities [3, 6, 7].

So far, there are several Coumarin based drugs are being used in clinics such as warfarin, phenprocoumon and acenocumarol. Coumarins also used as aroma enhancers in perfumes and cosmetics as well as industrial additives. In addition Coumarins have been studied to use as diagnostic agent, supramolecular medicinal drugs, pathologic probes and biological stains to monitor enzyme activity as well as pharmacokinetic properties in cells [3]. Especially, the large electron conjugated system in the coumarin's skekletal makes the compound to show wide range of properties. Recent study on the fluorescence property of coumarin based amine compound used as a dye to monitor siliceous structures in living organisms [8]. Coumarin scaffolds have been used as chromogenic and fluorogenic chemosensors for lethal cyanide ion [9]. Thus synthesis, extraction and analysis of coumarins for various applications have become an exceedingly attractive highlight.

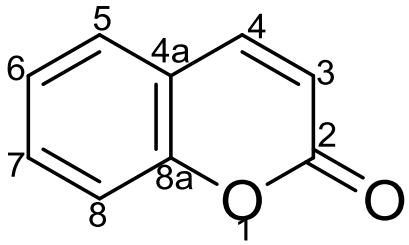


Figure 1. Chemical structure and IUPAC numeration of Coumarin.

Coumarins are known to have antioxidant activity [3, 10]. Antioxidants are molecules, have the potential to inhibit the oxidation of other molecules (reactive oxygen species) thus protect from the damages of high oxidative stress caused by reactive oxygen species. Reactive oxygen species (ROS) include free radicals and non-radical oxygen species which are highly reactive and generally formed by the partial reduction of oxygen. The high oxidative stress formed by the ROS causes deleterious effects to the human body. Finally inducing or causing harmful effects to the human body, such as cancer, coronary heart diseases, hypertension, inflammatory diseases, hemochromatosis, autoimmune disorders, and neurological disorder (eg. Alzheimer's disease, Parkinson's disease, etc.) [11-13]. Several researches have been carried out to study the antioxidant activity of various coumarins. They includes DPPH scavenging activity [14-16] ABTS assay [5,16] in vitro lipid peroxidation inhibition assay [16]. But it is curious to study the Hydrogen peroxide scavenging ability of the simple Coumarins. Because hydrogen peroxide (H_2O_2) is also a non-free radical ROS [17]. H_2O_2 also has the tendency to produce highly reactive hydroxyl radical, when it is partially reduced. Hydroxyl radical attacks the molecules on its path in human body and oxidize it into unstable radical. Thus it propagates a chain reaction and giving well significant contribution to the harmful effects of high oxidative stress.

Oxidative damage is also problematic in food products, cosmetic products, etc. It is one of the major cause of chemical spoilage (eg. Rancidity) which resulting in unfavourable changes in colour, flavour, texture and the nutritional value of the product, finally making question in the safety of the product [18]. Antioxidant can reduce the oxidative stress by acting as reducing agent to the ROS via donating its electron or Hydrogen. So, it's obvious that there is a need for the antioxidants to scavenge the ROS for the healthy life.

2- Materials and Methodology

2-1- Chemicals and Instruments

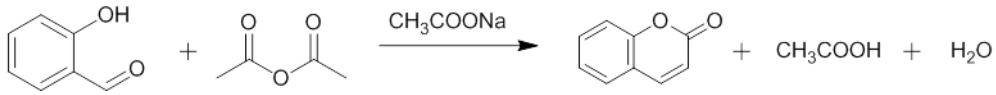
Standard chemicals of Sigma-Aldrich were obtained from chemical laboratory, Department of Chemistry, EUSL. The chemicals used were purified prior to its use. (Resorcinol was purified by recrystallization from Toluene; malic acid was purified by recrystallization from acetone and then ethyl acetate by adding petroleum ether (60-70 °C). Precoated TLC plates were used to monitor the reactions. Gallenhamp melting point apparatus was used to measure the melting points of the crystals. Infrared spectra were recorded on ATR– Thermo scientific Nicolet IS10 spectrometer. The ^{1}H spectra and ^{13}C spectra were recorded on Bruker NMR spectrometer (400 MHz) at room temperature (Methanol-d_4 as solvent). UV-Visible absorption spectra were recorded (as absorption versus wavelength) using Double beam scanning UV-Vis Spectrophotometer; BK-D580, in the range of 190-700 nm, by using 10mm Quarts cuvette.

2-2- Methodology

2-2-1- Synthesis of Coumarin

Salicylaldehyde (2.0 g), fused sodium acetate (2.5 g) and acetic anhydride (5.0 ml (0.052 mol)) were placed in three neck flasks. The mixture was refluxed gently for 6 hours at 180 °C and was TLC monitored. Calcium chloride drying tube was used at the top of the condenser. Then the mixture was steam distilled and the obtained residue was rendered

to basic with solid $NaHCO_3$. Then cooled, filtered and washed with cold water. The obtained crude coumarin was boiled in water with activated charcoal, filtered and concentrated. Then cooled product was collected and recrystallized from aqueous methanol [19].



Salicylaldehyde Acetic anhydride Coumarin

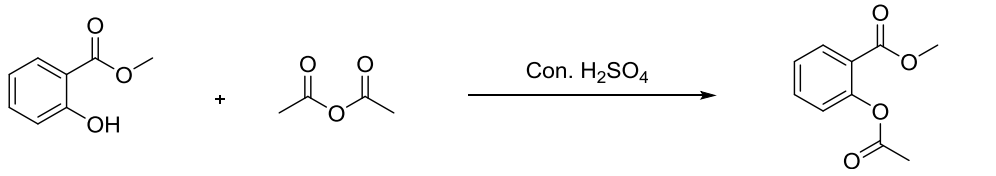
Figure 2. Reaction scheme for the synthesis of coumarin

2-2-2- Synthesis of 4-hydroxy coumarin

Part 1

Synthesis of acetyl methyl salicylate:

To a mixture of methyl salicylate (20 g) and acetic anhydride (20 g) concentrated H_2SO_4 (0.15 ml) was added and mixed thoroughly. Then the reaction mixture was allowed to stand at about room temperature for 40 minutes. Then the mixture was added to cold water (150 ml) and well stirred during the addition and after wards about half an hour. The resulting mixture was allowed to stand for 6 hours. The obtained solid product was filtered and washed with water. Then it was dried at room temperature in desiccator.



Methyl salicyclate Acetic anhydride Methyl O-acetylsalicyclate

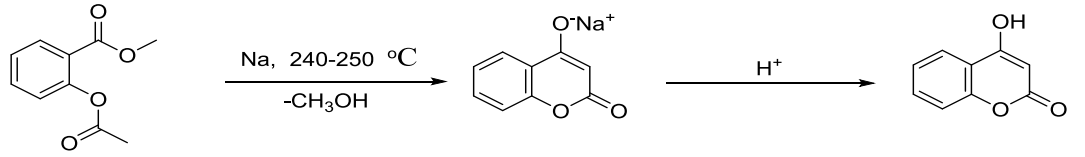
Figure 3. Reaction scheme for the synthesis of acetyl methyl salicylate

Part 2

Synthesis of 4-hydroxy coumarin:

Paraffin (30 ml) and metal sodium (2.4 g) were added into a three neck flask, equipped with a short fractionation column with condenser and was heated to 250 °C. Then dried acetyl methyl salicylate (20 g) was added portion wisely. Then the reaction mixture was maintained between 240-250 °C for 2 hours. Then it was filtered when the solution is hot. The obtained product was cooled and washed with low boiling petroleum fraction. The dried product was slowly added into the distilled water (100 ml) with vigorous stirring and maintained at about 60-70 °C. Then diluted HCl was added to reduce the pH to about 5.5-6.0. Flocculating gummy masses were removed by skimming and discarded.

The resulting mixture was shaken with equal volume of ethyl ether in a separating funnel. Then the aqueous phase was separated and acidified with diluted HCl to pH 1.5. The obtained crude 4-hydroxy coumarin was filtered and recrystallized from water twice [20].



Methyl O-acetylsalicyclate 4-hydroxy coumarin

Figure 4. Reaction scheme for the synthesis of 4-hydroxy coumarin

2-2-3- Synthesis of 7-hydroxy coumarin

Resorcinol (2.2 g) and malic acid (2.974 g) were added into concentrated H_2SO_4 (5.4ml) with stirring. The reaction mixture was maintained at about 120 °C till the effervescence ceased. Then the hot solution was poured into crushed ice with vigorous stirring. Then the mixture was allowed to stand for 24 hours and filtered [21]. The obtained crude product was recrystallized from aqueous ethanol twice. Then it was recrystallized from aqueous acetic acid.

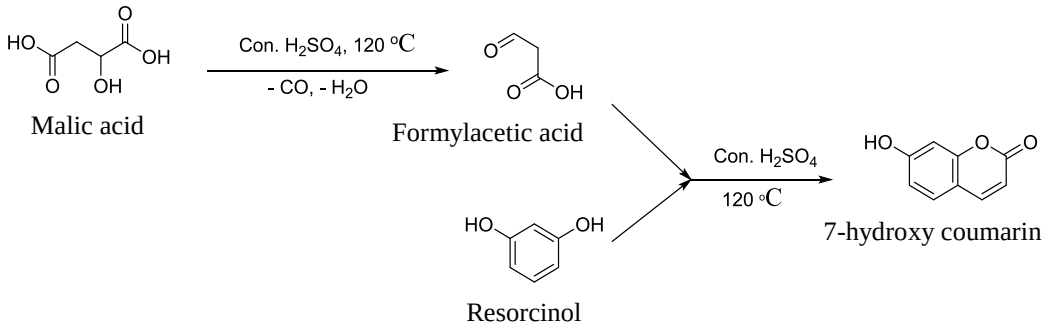


Figure 5. Reaction scheme for the synthesis of 7-hydroxy coumarin

2-2-4- Synthesis of 7-Hydroxy-4-methyl coumarin

A solution of resorcinol (1.0 g) in redistilled ethyl acetoacetate (1.305 ml, 0.0103 mol) was added drop wisely with stirring into concentrated H_2SO_4 (10 ml) and maintained between 5-10 °C. The reaction mixture was kept at room temperature for about 18 hours and TLC monitored. Then it was poured with vigorous stirring into a mixture of crushed ice and water. The obtained precipitate was filtered and washed with cold water. Then the precipitate was dissolved in 5 % NaOH solution and filtered. To the filtrate diluted H_2SO_4 (2M) was added with vigorous stirring until the solution become acid to litmus. The crude product was filtered and washed with cold water. Then it was recrystallized from ethanol (95 %) [22].

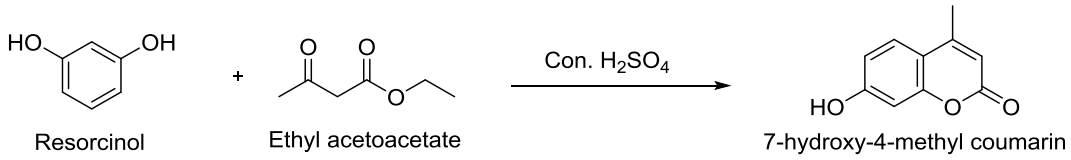


Figure 6. Reaction scheme for the synthesis of 7-hydroxy-4-methyl coumarin.

2-2-5- Measuring antioxidant activity (AOA)

Hydrogen peroxide (H_2O_2) scavenging activity:

The ability of the synthesized coumarin compounds to scavenge hydrogen peroxide was measured by using standard H_2O_2 scavenging assay method [23]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer saline (10x, pH 7.4). A series of various concentrated solution of each of the synthesized coumarin compounds (1000 ppm, 800 ppm, 600 ppm, 400 ppm, 200 ppm and 20 ppm) were prepared in ethanol (95%) and added (1 ml) to the hydrogen peroxide solution (40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution. Ascorbic acid was used as standard. All the experiments were carried out in triplicates in dark condition. The percentage of scavenged hydrogen peroxide was calculated by using the following equation,

Percentage of scavenged $H_2O_2 = [(A_i - A_t) / A_i] \times 100$

Where A_i is the absorbance of control and A_t is the absorbance of test.

H_2O_2 scavenging activity of the Coumarins was expressed in terms of IC_{50} value.

Ferric reducing antioxidant power (FRAP):

The antioxidant power of the coumarins based on the ability to reduce Fe^{3+} into Fe^{2+} was measured by using standard FRAP Assay method of Benzie and Strain (1999) [24]. FRAP working reagent was prepared from acetate buffer, pH 3.6 (3.1 g $CH_3COONa.3H_2O$ and 16 ml Con. CH_3COOH made up to 1 L), 10 mM TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) solution in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ solution in the proportion of 10:1:1 (v/v) respectively. The FRAP reagent was prepared freshly and was warmed to 37^0C prior to use. 0.1 ml of sample solution (1000 ppm) was added to the FRAP reagent (3.0 ml) and was undergone vortex. The reaction mixtures were incubated in water bath for 30 minutes at 37°C under dark condition. A solution, containing FRAP reagent without test samples was used as blank solution. Ascorbic acid was used for positive control. The absorbance of the samples was measured at 593 nm in UV-Vis Spectrophotometer. A standard calibration curve of Fe (II)-TPTZ complex was made by using a series of aqueous solution of $FeSO_4.7H_2O$. All the experiments were carried out in triplicates. FRAP value of the synthesized coumarin compounds was calculated by using standard calibration curve of Fe (II)-TPTZ complex ($y = 0.0253x + 0.0009$; $R^2 = 0.9959$) and was expressed as mmol Fe^{2+}/g of sample.

3- Results and Discussion

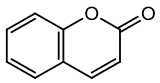
Characterization of the products

Coumarin:

IR (cm^{-1}): 1704 (C=O), 3057(C-H aromatic), 1277 and 1259 (C-O lactone's ester), 1618 (C=C), 1601 and 1562 (C=C aromatic).

UV-Visible (nm): 311 and 268 (n→π * of carbonyl chromophore and π→π* aromatic compound).

Melting point: 69 °C, Yield: 40%

Structure: 

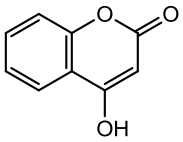
4-Hydroxy coumarin:

IR (v, cm-1): 3354 (O-H), 1660 (conjugated C=O), 1294 and 1273 (C-O lactone's ester), 1597 (C=C), 1553 (C=C aromatic).

^{1}H-NMR (CD_3OD, 400MHz) (δ, ppm): δ 5.65 (s, 1H, -C=C-H of lactone (3-H)), δ 7.92 (dd, 1H, ^{1}j=7.7 Hz, ^{2}j=1.5 Hz, 5-H), δ 7.64 (triplet of doublets, 1H, ^{1}j=7.7 Hz, ^{2}j=1.7 Hz, 6-H), δ 7.3638 (m, 2H, 7-H and 8-H).

^{13}C NMR (CD_3OD, 400MHz) (δ, ppm): δ 166.94 (4-C), 164.63(2-C), 153.82(8a-C), 132.54(7-C), 123.88(5-C), 123.19(6-C), 2 signals at 116.16 (4a-C and 8-C), 90.30(3-C).

Melting point: 214 °C, Yield: 21 %

Structure: 

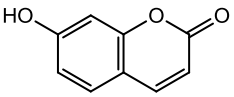
7-Hydroxy coumarin:

IR (v, cm^{-1}): 3199 (O-H), 1697(C=O), 1228 and 1257(C-O lactone's ester), 1602 (C=C), 1562 and 1516 (C=C aromatic).

^{1}H-NMR (CD_3OD, 400MHz) (δ, ppm): 6.19 (d, 1H, ^{1}j=9.3 Hz, 3-H), 6.72(dd, 1H, ^{1}j= 1.5 Hz, 8-H), 6.81(dd, 1H, ^{1}j= 8.4 Hz, ^{2}j= 1.5Hz, 6-H), 7.46 (d, 1H, ^{1}j= 8.4 Hz, 5-H), 7.86 (d, 1H, ^{1}j= 9.3 Hz, 4-H).

^{13}C NMR (CD_3OD, 400MHz) (δ, ppm): 162.27 (7-C), 161.73 (2-C), 155.84 (8a-C), 144.63 (4-C), 129.25 (5-C), 113.09 (6-C), 102.00 (8-C), 2 signals at 110.96 (4a-C and 3-C).

Melting point: 229 °C, Yield: 42 %

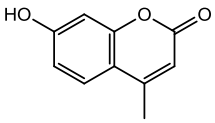
Structure: 

7-Hydroxy 4-methyl coumarin

IR (v, cm^{-1}): 3489 (O-H), 1674(C=O), 3095(C-H aromatic), 2817 (4-C-CH_3), 1276 and 1248 (C-O lactone's ester), 1598 (C=C), 1522 (C=C aromatic).

UV-Visible (nm): 323 and 295 (n→π * of carbonyl chromophore and π→π* aromatic compound).

Melting point: 186 °C, Yield: 83 %

Structure: 

H_2O_2 scavenging activity:

H_2O_2 scavenging activity of the simple Coumarins, C_1, C_2, C_3 and C_4 is shown in Figure 7, 8, 9 and 10 respectively. All tested simple coumarins has shown antioxidant activity to scavenge the H_2O_2. IC_{50} value of the simple Coumarins is shown in Table 1. IC_{50} value is the concentration of the sample required to scavenge 50% of H_2O_2. An antioxidant with lower IC_{50} value is the more potent antioxidant.

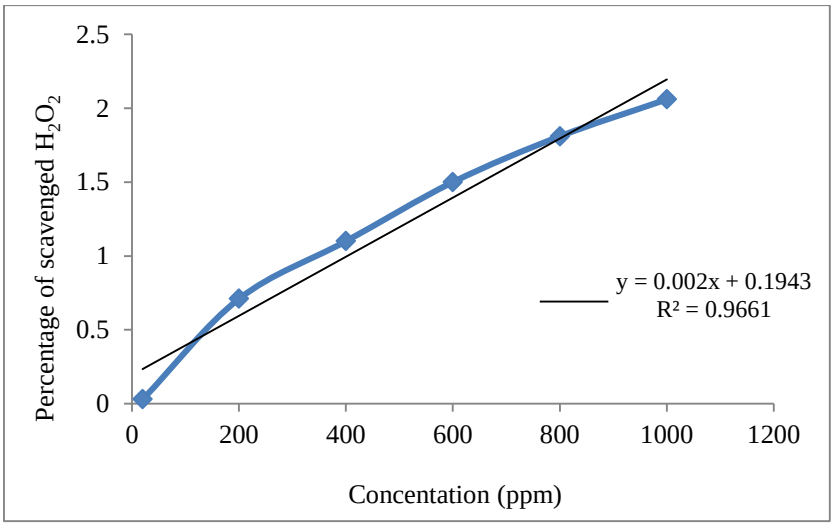


Figure 7. H_2O_2 Scavenging activity of C_1

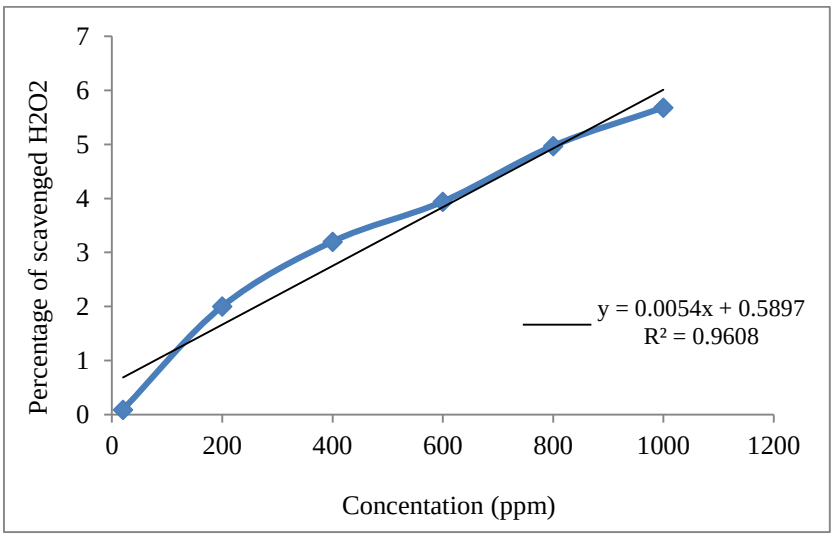


Figure 8. H_2O_2 Scavenging activity of C_2

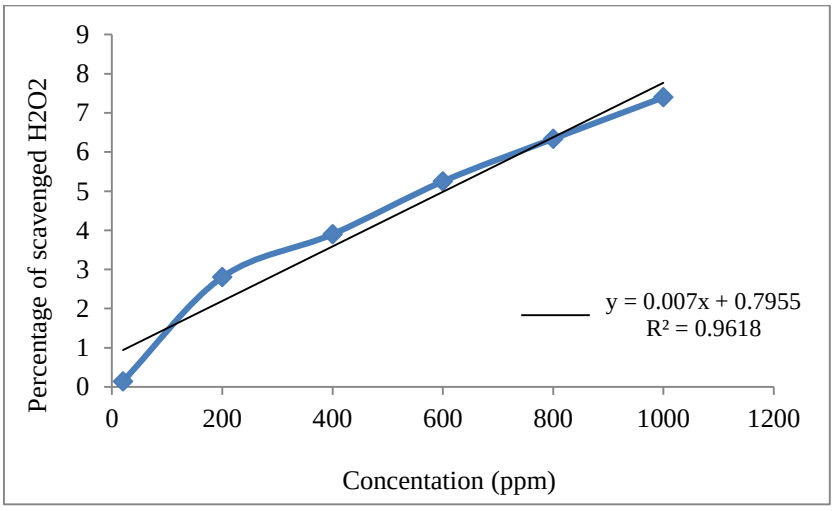


Figure 9. H_2O_2 Scavenging activity of C_3

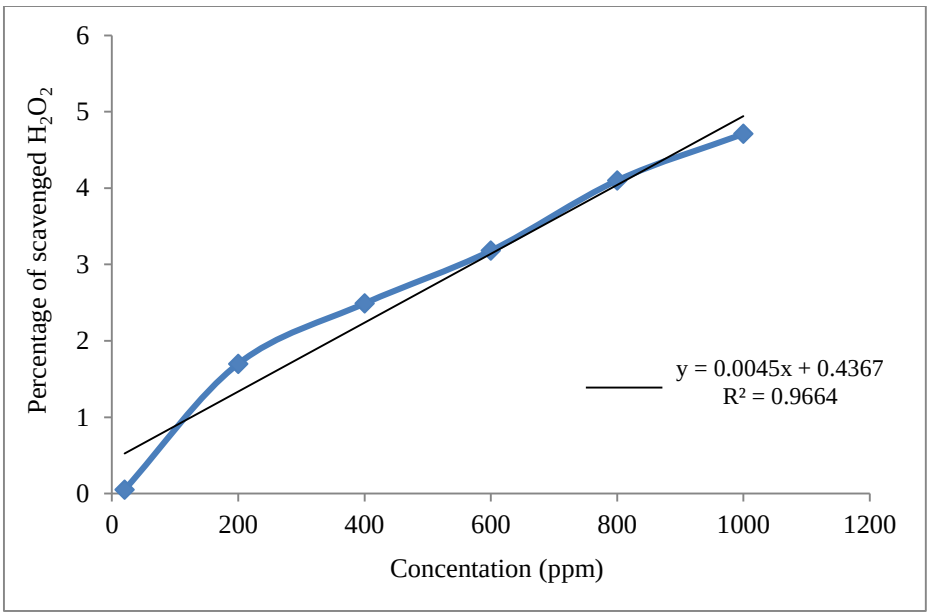


Figure 10. H_2O_2 Scavenging activity of C_4

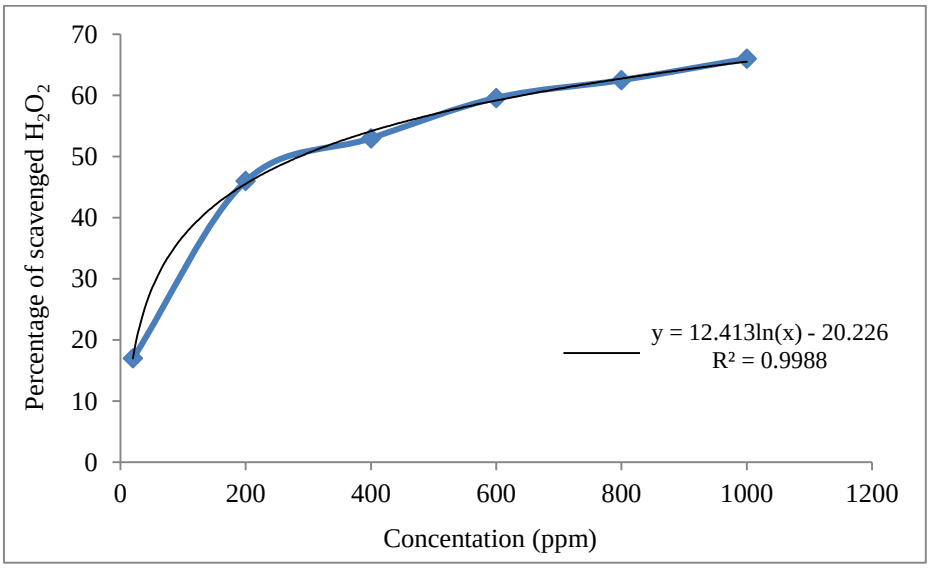


Figure 11. H_2O_2 Scavenging activity of Ascorbic acid

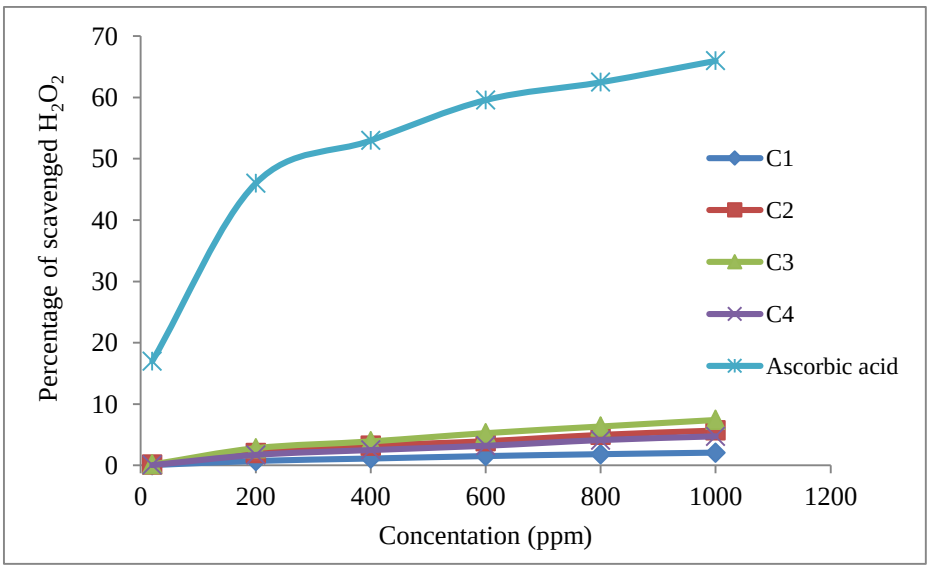


Figure 12. Comparison of H_2O_2 Scavenging activity of C_1, C_2, C_3, C_4 and Ascorbic acid

Table 1. **IC_{50} value for H_2O_2 scavenging activity of tested coumarins and Ascorbic acid**

Compound	H_2O_2 scavenging activity IC_{50} value (mg/dm^{-3})
Coumarin (C_1)	24902
4-hydroxy Coumarin (C_2)	9150
7-hydroxy Coumarin (C_3)	7029
7-hydroxy-4-methyl Coumarin (C_4)	11014
Ascorbic acid	286

The H_2O_2 scavenging activity of the four synthesized simple coumarins in decreasing order: $C_3 > C_2 > C_4 > C_1$. Hydroxyl coumarins (C_3, C_2 and C_4) have shown higher H_2O_2 scavenging activity than the parent compound C_1. Because the hydroxyl group in hydroxyl coumarins can act as proton donor thus it neutralizes the ROS. It donates the Hydrogen to the hydrogen peroxide and cleaves it into water molecules. Therefore the hydroxyl coumarins showed higher scavenging activity than that of coumarin. The reaction scheme for reaction between the hydroxyl coumarin and hydrogen peroxide is given in Figure 13.

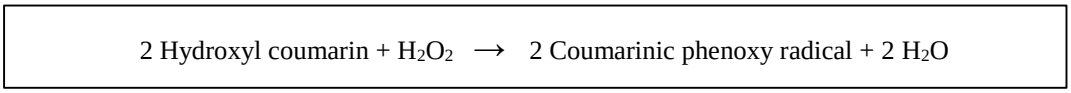


Figure 13. **Reaction scheme for the reduction of Hydrogen peroxide.**

Basically, the mode of action of hydroxyl coumarins could be similar to Glutathione, which is a cellular antioxidant found in our body that reduces H_2O_2 into H_2O by using its thiol functional group (Figure 14). Eventually the hydrogen donating hydroxyl coumarin is converted into a coumarinic phenoxy radical.

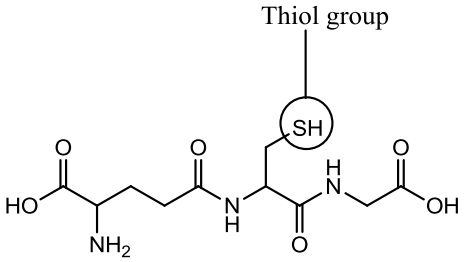


Figure 14. **Structure of Glutathione**

To be said as an antioxidant, the formed coumarinic phenoxy radical should be stabilized. If it is failed to stabilize the unstable radical then it may propagate a free radical mediated chain reaction which can lead to harmful effects in the human body. But stabilization of unstable free radical could be easily achieved in coumarins via electron delocalization as it is having the conjugated skeletal system. So the formed phenoxy radical undergoes electron delocalization on coumarin skeletal and makes the unstable radical into a stabilized form.

Among the four synthesized simple coumarins, 7- hydroxy coumarin (C_3) has shown highest H_2O_2 scavenging activity whereas 4-hydroxy coumarin (C_2) has shown the 2nd highest activity.

In both of these hydroxyl coumarins, the formed phenoxy radical undergoes for the delocalization on the coumarin skeletal system and get stabilized. But in case of C_3 the delocalization occurs in the benzene ring and also in the alpha pyrone moiety (Figure 15). In contrast of C_2 the delocalization of the formed radical occurs only in the alpha pyrone moiety (Figure 16). Thus C_3 phenoxy radical has an extended resonance stability than that of C_2. While comparing the number of resonance structures, C_3 phenoxy radical has higher number of resonance structures than that of C_2. When the number of resonance structure increases its stability also increase. Therefore, the extended resonance stability of C_3 makes the compound to show higher scavenging activity than that of C_2.

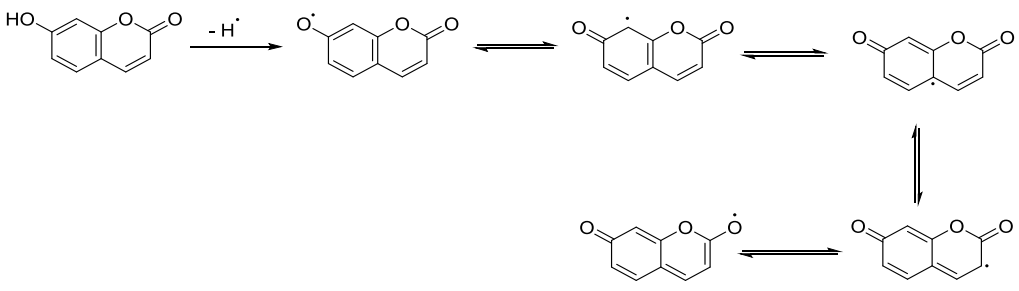


Figure 15. **Resonance stability of phenoxy radical of 7- hydroxy coumarin (C_3)**

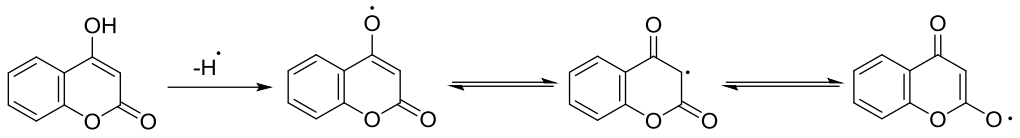


Figure 16. Resonance stability of phenoxy radical of 4-hydroxy coumarin (C_2)

However, the phenoxy radical of C_4 has more resonance structures than C_2, the scavenging activity of C_4 has been observed as lower than that of C_2. This could be due to the methyl group at 4th position that has negatively interfered on its antioxidant activity (Figure 17). However, all the synthesized simple coumarins have shown poor H_2O_2 scavenging activity when compared to standard- ascorbic acid (Figure 12).

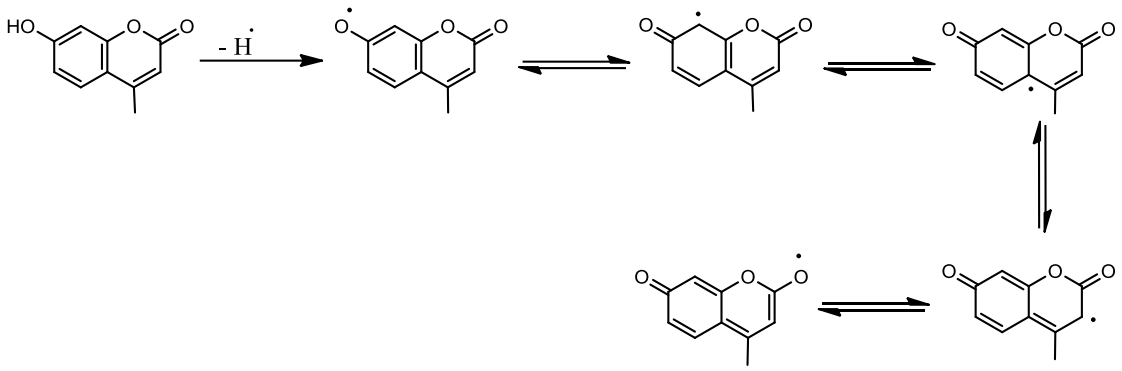


Figure 17. Resonance stability of phenoxy radical of 4-hydroxy coumarin (C_4)

The relationship between the scavenging percentage and the tested concentration of the simple coumarins; C_1, C_2, C_3 and C_4 is shown in Figure 7, 8, 9 and 10 respectively. All the tested coumarins showed a linear relationship (Y = mx + C) between scavenging percentage and the concentration of the coumarins. The coefficient of determination (R^2) of the linear relationship in C_1, C_2, C_3 and C_4 were 0.9661, 0.9608, 0.9618 and 0.9664 respectively. When increase the strength of the simple coumarins, its H_2O_2 sacavenging activity also increases linearly within the tested concentration (20 – 1000 ppm). Whereas the standard reference - ascorbic acid showed a logarithmic relationship of y = 12.413ln(x) - 20.226 with the coefficient of determination (R^2) 0.9988 Figure 11.

FRAP assay:

The total antioxidant capacity to reduce the Ferric ion into Ferrous ion can be measured by FRAP assay method which involves the reduction of Fe (III)-TPTZ complex into Fe (II)-TPTZ at low pH by an antioxidant where it acts as a reducing agent by donating single electron. Fe (III)-TPTZ complex is colourless at low pH but Fe (II)-TPTZ has intensive blue colour with λ_{max} 593 nm. The capacity of the antioxidant to reduce the Fe (III)-TPTZ complex into Fe (II)-TPTZ is directly related to the absorbance at 593 nm. Higher absorbance is positively correlated with higher FRAP value. The reduced amount of Ferric ion into Ferrous ion can be calculated from the standard calibration curve of Fe (II)-TPTZ complex. From which FRAP value can be calculated.

Table 2. FRAP value of tested coumarins, expressed in mean value ± SD

Compound name	FRAP value (mmol Fe^{2+}/g ± SD)
Coumarin (C_1)	0.014686 ± 0.000531
4-hydroxy Coumarin (C_2)	0.101035 ± 0.000708
7-hydroxy Coumarin (C_3)	0.200123 ± 0.001062
7-hydroxy-4-methyl Coumarin (C_4)	0.051137 ± 0.000885
Ascorbic acid	4.633979 ± 0.000708

The total antioxidant activity in terms of their ferric reducing capacity of the four synthesized simple coumarins in decreasing order: $C_3 > C_2 > C_4 > C_1$. The Ferric reducing ability order of the simple coumarins is very similar to that of the H_2O_2 scavenging activity order.

FRAP assay results reveal that compound C_3 has highest activity among the four simple Coumarins (Table 2). It has shown the FRAP value of 0.200123 ± 0.001062 mmol Fe^{2+}/g. But the compound C_1 has lowest FRAP value among the four simple Coumarins. C_3, C_2 and C_4 are hydroxylated coumarins. Their ferric reducing capacity is higher than that of

the parent compound - coumarin which has no substitutions. This indicates that the addition of hydroxyl group at 4th and 7th positions can increase the ferric reducing capacity of the coumarin. The second highest activity has been shown by C_2. Its activity is approximately half of the activity of C_3. This reveals that the hydroxyl group at 7th position is more effective to reduce the ferric ion than the hydroxyl group at 4th position in coumarin. When compared to C_3 and C_4, compound C_3 has approximately four fold of ferric reducing capacity of C_4.It indicates that the methyl group at 4th position can interfere negatively with the ferric reducing capacity of 7-hydroxy coumarin. But when compared to standard, ascorbic acid all synthesized simple coumarins (C_1, C_2, C_3 and C_4) have lower ferric reducing capacity.

4- Conclusion

Four simple coumarins have been synthesized and characterized by using UV, IR, ^{1}H and ^{13}C NMR spectra. All the synthesized coumarins showed antioxidant activity to scavenge hydrogen peroxide and to reduce ferric ion. The decreasing order of antioxidant activity of the simple coumarins is found to be 7-hydroxy coumarin, 4- hydroxy coumarin, 7-hydroxy for methyl coumarin and coumarin. This antioxidant activity order is similar in both cases of hydrogen peroxide scavenging ability and ferric reducing ability. Hydroxyl coumarins have higher activity than parent coumarin. Hydroxyl functional group shows an effective scavenging ability and increases the antioxidant activity of coumarin. Hydroxyl group at 7th position is more effective than that of 4th position on coumarin skeletal to scavenge the H_2O_2 and to reduce ferric ion. The 7-hydroxy coumarin has approximately two fold of ferric reducing ability of 4-hydroxy coumarin. The electron delocalizing system of coumarin ring plays a significant role on its antioxidant activity too. Methyl functional group at 4th position on coumarin ring system interferes negatively with the H_2O_2 scavenging activity and also with the ferric reducing capacity. However, when compared to standard reference- Ascorbic acid, the antioxidant activity to scavenge H_2O_2 and ferric ion of all the studied simple coumarins is poor.

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6- Conflict of Interest

The authors declare no conflict of interest.

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